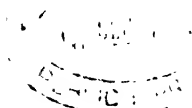




PRACTICAL BOTANY FOR BEGINNERS



REFERENCE

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PREFACE TO FIRST EDITION

THIS little book contains, in an abridged form, the elementary and more essential parts of the text of the larger *Course of Practical Instruction in Botany*. It is believed that beginners will find the directions sufficient to guide their first steps in laboratory work. The abridgment has largely consisted in the excision of supplementary descriptions of forms other than those which have been selected as the main types: in so far as that is the case, the publication of this book may be thought to encourage a narrow style of type-teaching.

Type-teaching in Biological Sciences appears at present to be inevitable in elementary classes, it lies chiefly with the teacher to avoid the evils which are apt to arise from it. In order to use this book with proper effect, his knowledge should extend far beyond the area of the work here specifically described, and the larger edition may help him towards this end. By grasping every opportunity of comparison of the type selected with allied forms which show differences of detail, he will then be able to guide the pupil to distinguish essentials from secondary details, and to check the dangerous tendency of beginners towards generalisation from too limited an area of fact.

F. O. BOWEN.

GLASGOW, *April* 1894

PREFACE TO SECOND EDITION

WE have found that the progress of Botanical Science, and especially of its terminology, has made a revision of this book necessary: accordingly we have endeavoured to bring the text up to date as regards terms, and at the same time we have rearranged the material, and introduced some additional examples, especially among the Flowering Plants. We believe that the book thus amended will serve better than before as an introduction to more extensive study.

F. O. B.

D. T. G.-V.

GLASGOW, *February* 1902.

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PRACTICAL BOTANY FOR BEGINNERS

The following is a list of apparatus required for ordinary work in the botanical laboratory. The articles marked with an asterisk () are absolutely essential to successful work.*

1. A pair of fine **scissors** with sharp points.
2. Fine-pointed **forceps**.
- *3. One or more **good razors** (see p. 7), and a strip and one for sharpening them.
4. **Scalpels** of various sizes: a fine eye-scalpel with a long arrow blade will be found to be very useful.
5. A **section-lifter**.
- *6. Mounted **needles**.
- *7. Several fine **camel's-hair brushes**.
- *8. **Watch-glasses** of various sizes, flattened at the middle the convex side so as to stand steadily.
- *9. Glass or porcelain **ointment pots**, with lids.
- *10. **Test-tubes** and **beakers**.
11. A **spirit-lamp**.
12. A **black enamelled tile** for mounting on.
- *13. **Glass slides**, with ground edges (3 in. × 1 in.).
- *14. Thin **cover-glasses**, square or circular ($\frac{7}{8}$ in. diameter).
- *15. **Blotting-paper**, cut or torn into small pieces.
- *16. **Drawing-paper** or **card**, with a hard smooth surface, or note-book of such paper, without lines.

*17 Hard **pencils** (H. or H.H.H.) and india-rubber.

*18 Gummed **labels** (1 in \times $\frac{3}{4}$ in) for naming slides.

*19 A coarse **duster**, and a finer **cloth**, e.g. an old pocket-handkerchief

20 A **rack** for keeping slides temporarily, and a **bell-glass** to cover it.

*21 A. **simple lens**.

*22. A **compound microscope**. This should be one of the smaller stands with a short tube such stands of varying merit are to be obtained from most makers. The microscope should be provided with—

*i. High and low **eye-pieces**: the longer is the lower power, the shorter the higher.

*ii. Two **objectives**, the lower power of about 1 inch focal length. The higher about **one-sixth inch** or **one-eighth inch** focal length.

iii. A **micrometer**, either adapted to the eye-piece, or a stage micrometer.

iv. A **nose-piece** to carry two, or, if necessary, more objectives: its use will save much time.

v. A **camera lucida** for drawing.

*23 A **rack** or tray to hold small **glass-stoppered** bottles containing **reagents**: the following are the reagents which are in most constant use—

*a. **Weak glycerine**, i.e. Price's pure glycerine diluted with an equal volume of distilled water.

*b. **Caustic potash**: make a 2 per cent. solution of the solid sticks of caustic potash in distilled water, and filter.

*c. **Acetic acid**: one volume of glacial acetic acid is to be diluted with 99 volumes of distilled water.

*d. **Iodine solution**: this may be obtained by diluting the *Liquor iodi* of the Pharmacopœia; or as follows: dissolve a small quantity of potassium iodide in distilled water, and add crystals of iodine: if the solution be too deeply coloured it may be diluted with distilled water to the colour of brown sherry.

*e. **Chlor-zinc-iodine** (Schulze's solution) may be purchased ready prepared from the dealers in

APPARATUS

micro-chemical reagents ; or it may be prepared as follows --

- (1) Dissolve 110 grms. of zinc in 300 c.c. of pure hydrochloric acid, and evaporate to 150 c.c. (sp. gr. about 1.8).
- (2) Dissolve 12 grms of KI in as little water as possible add 0.15 gm of iodine.
- (3) Mix (1) and (2), and filter, if necessary, through asbestos. The solution should have a dark sherry-brown colour.

*f. Solution of **aniline chloride**: a saturated solution is made in distilled water, filtered, and a few drops of hydrochloric acid added so that it may give a distinctly acid reaction. The solution should be colourless.

*g. A solution of **common salt**: a 5 per cent. solution, i.e. 5 grms. of salt to 100 c.c. distilled water.

Many other reagents besides these will be required for the work described below ; also substances for permanent mounting and sealing up of slides their preparation and uses are detailed in the Appendix A.

Care should be taken in the preparation of the reagents. they must be kept pure, and should be renewed occasionally. **Glass rods** with rounded ends are to be used for removing drops of the reagents from the bottles to the slide, and *the rod should always be cleansed before dipping it into a reagent bottle.*

*24. Two **wash-bottles** such as are in ordinary use in a chemical laboratory. the one should contain *alcohol* (methylated), the other *distilled water*.

I

A.—*Making Preparations*

I. **Preservation of Material.**—In many cases it is possible, and even preferable, to use fresh material, but it is often convenient to keep it for a time, since many of the specimens required are only to be obtained at certain seasons of the year: the best liquid for this purpose is ordinary methylated alcohol, in such quantity as completely to cover the material. It must be remembered that this will extract the green colouring matter (chlorophyll) from the material immersed in it, as well as resin and other substances.

II. **Hardening.**—For the general study of the histology of the mature parts of plants, it is often unnecessary to harden them, for the tissues are usually sufficiently firm to admit of their being cut satisfactorily. In the case of young, or of exclusively parenchymatous tissues, especially those of non-vascular plants, it is necessary to harden them, and for this purpose alcohol may be used.

When it is desired to study the structure of the protoplasm, and of the nucleus, special methods must be employed for hardening them, or rather for fixing them as nearly as possible in the condition in which they are during life. For this purpose one or other of the fluids mentioned below may be used. Care must be taken that the objects shall be of small size, that the quantity of hardening fluid be large relatively to the bulk of the object, and that the fluid have ready access to all parts of it. Large objects should be cut up into pieces of moderate size, so that the reagent may readily gain access to all parts of the tissue.

HARDENING

The following are the best fluids for this purpose :—

1. Absolute alcohol or methylated spirit.
2. Picric acid (saturated solution in water).
3. Chromic acid (0·1—0·5 per cent. solution in water).
4. Osmic acid (1—1·5 per cent. solution in water).

These reagents are only to be applied to fresh material.

When absolute alcohol is used, the object may be kept in it for an indefinite period. Such treatment generally makes the object brittle ; this may be remedied when the object is to be mounted in glycerine by placing it, for at least twenty-four hours before it is to be cut, in a mixture of glycerine and absolute alcohol in equal parts, leaving it exposed to the air so that the alcohol may gradually evaporate. The glycerine slowly saturates the object and restores its toughness. This can only be done when the sections are to be mounted in glycerine.

When picric or chromic acid is used, the object should be immersed in it until each part of it is thoroughly permeated by the reagent ; the length of time required for this varies with different material, and in the case of chromic acid, with the strength of the solution used, from a few minutes to twenty-four hours or more. The objects must then be washed thoroughly with water : they are then to be placed in dilute methylated spirit (50 per cent.), subsequently in stronger spirit (70 per cent.), and finally in absolute alcohol or strong methylated spirit, which must be changed so long as any colour is still extracted from the objects. They may be preserved in this for future use.

When osmic acid is used, the fixing effect is produced much more rapidly ; in the case of simple structures, such as unicellular or filamentous Algæ, a few minutes (5—15) generally suffices ; in the case of more complex structures, such as ovules, sporangia, growing points, &c., the object may be left in the acid till it looks black on the exterior : it must be then well washed with dilute alcohol (50 per cent.), and left in it for some time, and be then removed to 70 per cent. The sections are best mounted in dilute glycerine. In some cases osmic acid

PRACTICAL BOTANY

produces an excessive blackening of the cells, which can be removed by treatment with chlorine-water.

Of the hardening reagents above mentioned absolute alcohol, methylated spirit, and picric or chromic acids are those most generally used.

III. Cutting Sections.—In order to investigate the structure of the tissues of a plant or member, it is usually necessary to cut sections, *i.e.* thin slices, in various directions. To make a complete study of a solid mass of tissue, sections must be cut in three different planes at right angles to one another.

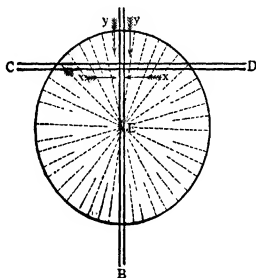


FIG. 1.

Taking the case of a cylindrical stem, the best way to study its structure would be to cut—

(i) **Transverse sections**, in planes at right angles to the organic axis.

(ii.) **Radial longitudinal sections**, in longitudinal planes including the organic axis

(iii.) **Tangential longitudinal sections**, in longitudinal planes which do not include the organic axis.

This may be illustrated by a diagram (Fig 1), which may be taken to represent the transversely cut end of a cylindrical stem, the tissues being arranged with reference to a central point (E): **transverse** sections are those which are in transverse

CUTTING SECTIONS

planes, parallel to the plane of the paper in Fig. 1. The line including the central points of successive imaginary transverse sections is the organic axis.

Radial and tangential sections are both in planes vertical to that of the paper in Fig. 1. a **radial** section (A E B) includes the organic axis (E), and a slice of tissue thus cut when examined from a direction indicated by either of the arrows (x) will show in surface view those cell-walls which run radially. a **tangential** section (C D) does not include the organic axis (E), and such sections when examined from a direction indicated by the arrows (y y) will show the tangential walls in surface view, while the radial walls, previously seen in surface view, would present their cut edges to the observer.

In the case of tangential sections only the central part of the section (*i.e.* the part near to y y) is to be examined, for obviously in the more lateral parts of the section (C D) the radial lines are not cut vertically but obliquely.

In all cases the sections must be cut accurately in the plane intended: if the sections be cut obliquely the difficulty of understanding the structure will in almost every case be enormously increased.

A **razor** of good quality is the best cutting instrument: there is some variety of opinion as to the best form of blade; some prefer a hollow-ground razor, which, though well suited for cutting small sections, will not serve for sections of large area; for such work a razor with one flat side is recommended. For general use, not only in cutting small objects and soft tissues, but for the every-day work of the laboratory, an ordinary, very slightly hollow-ground razor of good quality will be found the most useful. The razor should be stropped to a keen edge, and the blade should be carefully protected when not in use. it should never be left open on the work-table, and the blade should always be cleaned after use, since the acid juices of plants are apt to corrode it. It will be found convenient to have a glass of water (or weak spirit when resinous tissues are being cut) on the work-table, into which the blade of the razor may be plunged at once after use; this will prevent immediate corrosion.

The success of work in the laboratory depends very greatly on due care in the direction of section, and on the condition of the edge of the razor.

All the sections required in the succeeding pages of this book can be made by hand: elementary students are advised to avoid the use of a microtome; they should cultivate that small amount of manual dexterity which will suffice for the successful preparation of such objects as will be described below.

When cutting sections the object to be cut may be held in the thumb and first finger of the left hand, while the razor is held firmly by the right: The edge of the razor is not to be rudely forced through the tissues of the specimen, but a sliding cut is to be made, thus using a considerable length of the edge of the razor. in this way a smoother surface of section is obtained, and the tissues are not displaced as they otherwise might be.

Care must be taken to keep the object and the razor wet during the process of cutting, in order to avoid the entrance of air into the tissue, and to prevent adhesion of the section to the razor. When fresh material is cut, water or very dilute alcohol may be used for this purpose, but if material which has been hardened is cut, it is advisable to use alcohol of the same strength as that in which the material has been preserved

IV. Embedding.—The objects are frequently so large that they may be held in the hand whilst they are being cut. If they are too small for this, it is convenient to embed them in some substance.

The simplest method is to fix the object into a slit in a piece of pith. Dried elder-pith is the best, and it may be bought ready prepared from the dealers.

When the object to be cut is small, or easily damaged, it is more convenient to embed in some easily fusible substance: by this means also the form of the object is less likely to be distorted in the process of cutting. Various substances, or mixtures of substances, are used for this purpose, of which soft paraffin is perhaps the best. Samples of paraffin which vary in hardness and melting point may be obtained from the dealers.

The ordinary method of embedding is to make a cavity in a piece of the substance sufficiently large to contain the object, which, if fresh, must have been previously washed with alcohol to remove all traces of water from its surface. If the object had been previously preserved in alcohol, all superfluous fluid must be removed from the surface with blotting-paper, but care must be taken that the spirit which permeates the tissue shall not evaporate. The object is then placed in the cavity, and without unnecessary delay a small quantity of the embedding substance, melted over a spirit lamp in a small tinned iron spoon, is poured into the cavity so as to surround and cover the object.

If the object be small, it will be found convenient to heat one end of a thick copper or platinum wire, and with it melt a small cavity, in which the object may be placed in such position as is found convenient.

The sections must not be made until the paraffin is quite cold, and firmly set.

It is important to keep the embedded objects wet with alcohol during the process of cutting, in order to prevent the drying-up of the object, and its consequent contraction away from the substance in which it is embedded.

The sections when cut should be removed at once to a watch-glass containing alcohol or water, by means of a camel's-hair brush, or a jet of alcohol or water from a wash-bottle. the thicker sections may then be removed, and the thinnest ones selected for observation.

V. Mounting Objects.—Various specimens, whether sections or objects which may be examined whole, require varied treatment, and the common methods in ordinary use will be described below, and illustrated by experimental exercises (see pp. 18, &c.); meanwhile a few practical suggestions will be given which are to be observed in all cases, whatever the special method of treatment may be.

1. *Study to avoid all unnecessary manipulation of specimens; never apply a reagent at haphazard, but only when you have a definite purpose for doing so.*

These rules apply specially to staining reagents, which

should only be used when their assistance is actually required. the primary end of the anatomical investigations detailed below is not to prepare a number of objects pleasing to the uneducated eye, but to gain a knowledge of the structure of the plant-body as it is in the living state, and this end may as a rule be best attained by the simplest methods.

2. See that the glass slide and the cover-glass are *perfectly clean* and dry, and show a bright polished surface before using them; they should be cleaned immediately before use, and after cleaning, their surfaces should not be touched with the fingers, nor should the cover-glass be laid flat on the table, but tilted on its edge

If difficulty be found in cleaning glass slides or covers with water, a drop of weak acetic acid will be found effective.

3. In mounting, whatever the fluid may be, take *only so small a drop of it as shall just suffice to fill the space between the slide and the cover-glass, and extend to the margin of the cover*: judgment as to the quantity necessary can only be acquired by practice. If too much fluid has been used the excess must be soaked up with slips of blotting-paper, or filter paper.

4. *The practice of scrupulous cleanliness cannot be too strongly impressed upon students as the basis of all successful work with the microscope*, and it is in the use of fluid reagents that the greatest care is necessary; if too large a quantity be used it is apt to extend to the lower surface of the slide, and so to the stage of the microscope; or to be smeared over the upper side of the cover-slip, and may then gain access even to the objective; *it is absolutely necessary that both the front lens of the objective, and the upper side of the cover-slip be perfectly clean and dry, also the lower surface of the slide and the stage of the microscope*

5. Having taken a sufficiently small drop of the mounting medium, and having placed the object in it, bring down the cover-glass *obliquely upon the drop so that one edge of it is first wetted by the medium*, then let down the slip gently, so as to allow the medium time to spread out under the cover-slip; this may be done either by holding the cover-slip in a pair of clean forceps,

MOUNTING

or hold the slip by its edges in an oblique position in the finger and thumb of the left hand, while it is supported by a clean needle held in the right; then, the lower side being wetted with the medium, gradually withdraw the needle and thus gently lower the slip. It would be well at first to practise thus lowering the cover-glass over a drop of water, so as to acquire judgment of the quantity of fluid required, and skill in avoiding the inclosure of air-bubbles.

6 One great purpose of the above directions is to avoid the presence of **bubbles of air** in the medium surrounding the object; their presence is one of the great difficulties of the beginner, who is therefore advised, in his own interest, to follow carefully the directions above given. In some specimens, especially when fresh, air-bubbles will be found entangled in the tissues, or attached to the outside. A good method for avoiding them in mounting fresh material is to moisten with **alcohol** (weak alcohol will do) for a few seconds before mounting: by this means the surface of the object will be more thoroughly wetted than would otherwise be the case. Obstinate bubbles may be expelled by heating over a spirit-lamp, but as many objects will not stand such rough treatment, a better method is to exhaust them under the receiver of an air-pump.

7 After an object has been mounted it is often necessary to apply to it certain staining, or micro-chemical reagents. This may frequently be done, without removing the object from the slide, by **irrigation**: successive drops of the reagent are placed on the slide, close to one edge of the cover-slip, *special care being taken that the fluid does not spread to the upper side of the cover*, while a small piece of blotting-paper is pushed up to the opposite edge of the cover-slip, so that, when it comes in contact with the medium in which the object is mounted, it will soak it up: the space thus vacated by the medium is taken by the reagent, and if the latter be supplied in sufficient quantity, a stream of it will pass under the cover-slip and bathe the object. It is obvious that for such a treatment to be successful the medium and the reagent must be fluids which will mix. Students are warned against too readily

PRACTICAL BOTANY

accepting negative evidence as the result of observations by irrigation : the reagent may frequently pass under the cover-slip without permeating the object, or the edges only of a section may be affected : in order to insure the object being bathed by the reagent, it is well to raise the cover-glass gently with a needle, or even to raise the section itself slightly with the point of a needle.

8. *Never use more than one cover-slip on a single slide, though several objects may, if small enough, be covered by one slip : the cover-slip should be as nearly as possible in the middle of the slide.*

9. *Pressure should never be laid on the cover-slip, except in certain special cases : a bad section will not be improved by being squeezed flat, while a good section may be easily rendered worthless by such treatment.*

10. Before putting a slide aside for subsequent observation *be sure that the medium used is one which will not evaporate*. alcohol, water, and solutions in water, such as iodine solution, aniline sulphate, salt solution, are all liable to evaporation, while chlor-zinc-iodine and glycerine are not.

11. Before putting a slide aside *be careful to label it*, writing at once on an adhesive label the *name of the plant, part, direction of section, and medium in which it is mounted.*

B.—Adjustment of the Microscope for Work.

Before beginning work it should be ascertained *that the microscope is in good working order* : if it be a stand without rackwork, see that the stage of the microscope is clean, and that the tube moves easily and smoothly ; if it does not, take out the tube, and rub it with a clean cloth ; if still stiff, apply a very little mineral oil or vaseline, and rub with a clean cloth till there is no appearance of oil on the tube : it should then work freely. Nothing causes a greater strain on a microscope than neglect of this simple point.

Next *see that the lenses are clean* ; to this end first dust the mirror, and adjust it so as to reflect light through the instrument : insert first one eye-piece, then the other : rotate each eye-piece,

ADJUSTMENT OF MICROSCOPE

and if any specks be seen to rotate with it they are on the lenses of the eye-piece, and must be removed with a soft chamois leather, or a fine linen or silk cloth (old pocket-handkerchief). Carefully examine the front lens of each objective, and if any dirt be seen wipe the lens *gently* with a chamois leather, or fine linen or silk cloth. Glycerine is apt to gain access to the objective in careless hands ; when this is the case the lens is to be washed with a jet of distilled water, and carefully dried. Since the lenses are often fixed with balsam, great care must be taken that they should not be smeared with Canada balsam, or Dammar · when this has happened the lens should be gently rubbed with a cloth wetted with a very little benzol, or alcohol. In all cases the cleaning of lenses should be carried out as gently as possible, to avoid destroying their polish.

The best light for microscopic work is that reflected from white clouds in a northern sky, and a window with a northern aspect should be selected. *Never use direct sunlight, and avoid using artificial light*. If the only available room has a south aspect, a white blind is to be used, so as to cut off direct sunlight, or a piece of white card may be fitted to the surface of the mirror, so as to act as a less perfect reflector.

The body of the microscope should be vertical ; with the short microscopes now in use, the oblique position is quite unnecessary, and very inconvenient when mounting in fluid media, or irrigating with fluid reagents.

Always examine an object with a low power first, and afterwards, if necessary, with a higher power. It is a general principle of microscopic practice that observations should be made with the lowest possible power sufficient for distinct vision. *Never use the high power unless the object be covered with a coverslip.*

When a low power is used a larger hole of the diaphragm below the stage is to be placed opposite the aperture in the stage ; when a high power is used a smaller hole of the diaphragm is necessary, otherwise the definition will not be satisfactory.

Some difficulty will be felt at first in finding the focus. There are two adjustments of focus—the coarse and the fine : the latter

is never to be used until the *focus* is approximately found with the coarse adjustment. *The coarse adjustment* is effected by a sliding tube in the smaller microscopes in general use by students having drawn this tube out, screw on the *low power objective* (the one with the larger front lens, focal length about 1 inch from object), then replace the tube so that the objective is about $1\frac{1}{2}$ inches from the stage, and having adjusted the mirror so as to illuminate the whole field, place some object on a slide at the centre of the stage; hold the slide with the thumb and forefinger of the left hand, while the upper end of the tube is grasped with the right; then slide the tube gradually downwards with a spiral movement until the object comes dimly into view; then begin to use the *fine adjustment*, which is worked by a screw with a milled head, the position of which varies in different instruments: this head is to be turned so as to lower the tube, when the object will become clearer, and ultimately be in perfect focus. The focus is to be found in the same way with the *high power*, but in this case greater care is necessary, since when in focus the objective is nearer to the object in careless hands the position of focus is apt to be over-stepped, and the objective advanced so as to touch, or even crush, the object; *this is to be carefully avoided*, as it not only damages the object, but may also ruin the objective.

When the focus has been found, the fine adjustment is to be worked constantly up and down by the right hand during observation; by this means a series of optical sections of the object is brought successively into view, and in this way the observer builds up mentally a conception of the object as a solid body. In so far as an objective lends itself to this it is said to possess good *penetration*. Meanwhile the forefinger and thumb of the left hand will be at liberty to move the slide on the stage so as to bring into the field of view different parts of the preparation.

Observers should accustom themselves to using both eyes indifferently, and when one eye is being used for observation, the other should be kept open: a little practice will soon overcome any difficulty which may be at first found in doing this.

Care is necessary in removing the slide from the stage, especially when the high power has been used: in this case the

DRAWING

Tube should first be raised so as to remove the objective from close proximity to the stage, and the *slide should then be slipped off the stage*, not lifted off. Want of attention to these points is apt to result in smearing the objective with glycerine, or other media.

Drawing from the Microscope.—Nothing compels attention to details of an object so successfully as drawing it; while, as it is impossible to make a drawing of an ill-prepared object, the intention to make a drawing will have its effect upon the care devoted to preparation and mounting. *It should be a rule for students to draw every object they observe*, not merely for the sake of the drawings as memoranda, but in order to acquire a habit of close observation.

For drawing, a hard pencil (H H H) is recommended, and it must be cut to a fine point: paper with a hard smooth surface is to be used, or better, a thin drawing card or Bristol board with a hard surface. A decisive style of drawing should be adopted, in which every line is clear and conveys its own meaning.

For ordinary purposes a freehand drawing will suffice, the scale being as nearly as possible that of the object as it appears under the microscope, or larger, if the object be a complicated one. whatever the scale, the *proportion* of the several parts is to be scrupulously followed. Coloured chalks, or better, light washes of water-colour, may be used for distinguishing tissues of different character, and in making a series of drawings the same colours should be assigned to corresponding tissues throughout the series.

When drawing cell-walls of appreciable thickness, they should be indicated by a double line; solid bodies should be shaded so as to give the idea of light coming from one side, and in a series of drawings the side selected should be maintained throughout.

Measurement of Objects—Measurements may be most readily made by means of an **eye-piece micrometer**, which is a glass slip, fitted into the eye-piece, and having a scale engraved upon it. The value of the divisions of this scale varies with the combination of lenses used; accordingly, before the

micrometer can be employed in the actual measurement of objects, the value of the divisions must be determined for each combination, and a table of the results should be kept for reference in the case of the microscope. To determine the value of divisions of the scale under a given combination of glasses, a stage micrometer, having lines drawn to $\frac{1}{1000}$ ths of an inch apart, should be placed on the stage, and focussed under the objective and eye-piece whose magnifying powers it is desired to measure: the relation of the divisions of the stage micrometer (these intervals being of known value) to those of the eye-piece micrometer is then to be noted. Suppose that the interval between two lines of the stage micrometer covers the intervals between six lines of the eye-piece micrometer, the former being $\frac{1}{1000}$ th of an inch apart, the interval between two lines of the latter (with that combination of lenses) will correspond to $\frac{1}{6000}$ th of an inch, and the linear measurement of any object which fills such an interval under that combination of lenses will be $\frac{1}{6000}$ th of an inch. It is usual to state the size of objects seen under the microscope according to the linear measurement of the diameter. The simpler method of measurement by laying the stage micrometer inverted on the slide carrying the object to be measured, though direct, is open to many objections, and can at best only be used with low powers.

C.—First Practice in Mounting and Observation.

Scrape the freshly-cut surface of a Potato-tuber lightly with a knife, and place a small quantity of the scrapings in a drop of water in the middle of a glass slide.

Mount carefully according to the directions given under head (V.) on p. 9.

Having put on the low power, follow the directions given on p. 12, &c., for finding the focus.

Observe scattered through the water a large number of somewhat ovoid, colourless, bright-looking bodies. These are starch-grains.

Having practised finding the focus under the low power

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several times, screw on the **high power**. advance the objective slowly and carefully, until the objects come into view, and observe that the starch-grains show a **stratified structure**; they are usually slightly pointed at one end, near to which is a round clear spot—the **hilum**. These details will be more easily seen if a small hole of the diaphragm be used.

To practise irrigation (see p. 11, par. 7), introduce under the cover-slip a single drop of iodine solution, drawing it in by means of blotting paper.

Note the advance of the pale yellow solution under the cover slip, and its effect in staining the starch blue.

II

PRACTICAL EXERCISES ON THE STRUCTURE OF THE VEGETABLE CELL INVOLVING SIMPLE METHODS OF PREPARATION.

Before entering upon the work described below, the preceding pages should be carefully read through, otherwise the beginner will be apt to make serious mistakes in manipulation.

NOTE —The method of mounting fresh material in water will be used throughout this chapter: it has the following advantages —

1. It is the simplest possible
2. The cells are seen unaltered, *i.e.* in the living state, and it is thus specially suitable for observations on fresh material.
3. When the living cell is thus mounted the effect of any reagent soluble in water may be observed by irrigation: thus it is the natural starting-point for the study of the micro-chemical reactions of the living cell.

It is however open to objection on the following grounds:—

1. The slides thus prepared cannot be kept, since the water would evaporate.
2. The refractive index of water being relatively low, the objects do not appear so transparent as in more highly refractive media.
3. Bubbles of air are very apt to be included with the object

1. Take a young leaf of *Primula sinensis* (common Primul of greenhouses): with a knife scrape off from the petiole

PRACTICAL EXERCISES

number of the hairs which project from its surface ; moisten them with alcohol to remove the oily secretion, and mount quickly in water. For method of mounting see (5), on p. 10. Examine first with a low power, and observe the hairs as a whole, each consisting of several cells attached end to end, and with transverse septa dividing each cell from its neighbour.

Examine now with a high power, and observe the following parts of each cell, selecting first the larger cells for examination, which form the lower part of the hair.

1. The **cell-wall**, a definite layer which forms a complete outer covering of each cell, and is continuous between them, forming the septa.

2. A film of colourless granular material, the **protoplasm**, which forms a continuous and complete lining to the cell-wall internally, and surrounds a large central cavity—the **vacuole**—filled with transparent **sap**.

3. Embedded in this are more or less numerous **chlorophyll-corpuscles**, or **chloroplasts**, of small size, oval outline, and pale green colour. Also—

4. In each cell a **single nucleus** may be found. It is an oval body, of pearly appearance, and usually lies laterally, embedded in the protoplasmic film. As its position is not definite, and it has no colour, it is sometimes difficult to find it without the help of a stain.

Irrigate (see p. 11) the slide with *iodine solution*, and note the following results :

1. The cell-wall is unaltered in form, and is not stained appreciably.

2. The **protoplasm** will be seen to be coloured a more or less deep yellow or brown. It may also separate from the cell-wall and contract, so that it can now be better seen as a distinct film.

3. The **chloroplasts** will be a dusky brownish purple.

4. The **nucleus** will have been brought into prominence by its staining more deeply yellowish brown than the protoplasm which embeds it ; while frequently within it will be seen a deeply coloured granule—the **nucleolus**.

PRACTICAL BOTANY

II. Examine specimens of the green filamentous Alga, *Spirogyra*.

This Alga is commonly to be found in summer, growing in stagnant, or slowly flowing, fresh water. It is to be recognized by the bright green colour of the unbranched filaments, which are irregularly coiled together, and of sufficient size to be distinguished by the naked eye: the whole flocculent mass feels slimy when lifted from the water.

Mount a *small* quantity of the *Spirogyra* in water: examine it first under a low power, and observe on one of the largest specimens—

1. That the cylindrical filament is limited by a definite **cell-wall**.

2. That transverse partitions—**septa**—which are continuous with the limiting cell-wall, divide the filament into a linear series of **cells**.

3. That each cell contains a **protoplasmic body**, the most marked part of which will be one or more **spiral chloroplasts**, coloured bright green by chlorophyll.

In order to study the structure of the protoplasmic body in detail, put on a high power, and, focussing carefully, make the following observations on the filament, which, it is to be remembered, is in the living condition.—

1. Having recognized the smooth colourless **cell-wall**, note that it is closely invested internally by—

2. A continuous film of colourless **protoplasm**.

3. That this protoplasmic film surrounds a large central cavity—the **vacuole**—filled with perfectly transparent **cell-sap**.

4. That in the peripheral film are embedded one or more **green spiral bodies (chloroplasts)** of flattened form, and very **irregular margin**, each including numerous lenticular, highly **refractive bodies, the pyrenoids**.

5. Focussing carefully downwards into the central cavity, a highly refractive, colourless, lens-shaped body is to be seen suspended in a central position by numerous finely granular **protoplasmic threads**: this body is the **nucleus**.

- a. These several points should be made out in the living cell, without treatment with any reagent, but their observation

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may be made easier in various ways. Irrigate (p. 11) with *iodine solution* and observe the following results of that treatment :—

1. The cell-wall will not be appreciably altered or stained.
2. The film of protoplasm ("primordial utricle") will have stained a pale yellow or brown, and may often be seen to have separated partially or completely from the cell-wall, from which it may now be readily distinguished.
3. The chloroplasts will have assumed a dusky colour, while the pyrenoids will be a dark purple.
4. The nucleus will have stained a deep yellow or brown, and inclosed within it one or sometimes two **nucleoli** may be recognized by their deeper colour and high refractive power.

b. Irrigate a fresh specimen with 5 per cent solution of *common salt*, and watch the result; which may take some minutes to appear. The protoplasm will gradually separate from the cell-wall, and while the latter retains its form and position, the protoplasm will contract, and rounding itself off, ultimately appear as a more or less irregularly oval or spherical body.

c. Mount a fresh specimen in water, and irrigate with *glycerine*; the cells will collapse, owing to the sudden abstraction of water of the cell-sap.

d. Mount a fresh preparation in water: irrigate with *potash* solution, and observe that the protoplasmic contents swell and lose their definite outline, and the whole becomes more transparent.

III. The above examples show cells aggregated in a linear series, constituting a filament. the next specimen illustrates the more complicated arrangement of cells in two dimensions of space, *i.e.* as a flat plate of tissue. Mount a small prothallus of a Fern (or the thin lateral part of a large one) in water, and examine it under a low power such Fern prothalli are commonly to be found on the damp surface of soil or flower-pots in ferneries where the air is constantly damp: or they may be readily grown by sowing Fern-spores on moist soil.

It will be at once obvious that the thin plate of tissue, one layer of cells in thickness, is partitioned off into a number of **cells** of polygonal form, which are in close connection with one another, so that no spaces intervene between them. Note the green granules (**chlorophyll-corpuscles** or **chloroplasts**), which are here to be seen in considerable numbers in each cell.

Examine the preparation under a **high** power, and distinguish—

1. The **cell-walls**, which are thin, highly refractive, and of almost uniform width throughout: the extreme margin of the prothallus will be found best adapted for their observation.

2. A colourless film of granular **protoplasm** ("primordial utricle"), which is in close apposition to the cell-wall, and surrounds a large central cavity (the **vacuole**) full of colourless **cell-sap**: in this protoplasmic film are embedded—

3. The **chlorophyll-corpuscles** or **chloroplasts**, which will now be seen to be flattened disk-like bodies.

4. A single, highly refractive **nucleus** is to be found in each cell, its position being variable.

- a. Treat the preparation with *iodine* solution, and observe that—

1. The protoplasm will be stained brown.

2. The chlorophyll-corpuscles, for the most part, a dusky purple.

3. The **nucleus** will be more deeply stained, and will accordingly be more easily recognized. One or more roundish, highly refractive bodies may be seen in the nucleus (**nucleoli**).

4. The cell-walls are not stained.

- b. Treat a fresh preparation with potash solution, and warm gently over a spirit-lamp: observe that the protoplasm, chlorophyll-grains, and nucleus lose their definite outlines, and, undergoing a process of swelling, become at the same time more transparent. This may best be seen in a specimen which has been bleached in alcohol.

- c. Mount another preparation in "eau de javelle" (see Appendix A), and observe it at intervals for some minutes: a similar result will be seen, viz., the contents of the cells swell, and the whole tissue becomes more transparent: this is

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especially the case in the region near the apex of the prothallus.

d. Irrigate a fresh specimen with a 5 per cent. solution of common salt, and watch the result: it will be seen that the protoplasm contracts, often taking the form of an almost spherical ball, thus separating from the cell-walls with which it was originally in contact: the latter will now appear as a continuous network of partitions dividing the whole prothallus into a number of chambers.

If this preparation be examined under a high power, a number of delicate protoplasmic filaments may be seen connecting the outer surface of the contracted protoplasm with the cell-wall. This indicates that the two bodies are not merely in apposition in the living cell, but are closely connected.

A cell in this state is said to be **plasmolytic**: the contraction is due to the withdrawal of water from the cell sap by the salt solution, this withdrawal not being compensated for by the entrance of salt solution into the vacuole. The salt solution diffuses through the cell-wall, and occupies the space between the cell-wall and the contracted protoplasmic film, but it cannot pass through the protoplasmic film to any considerable extent.

On washing the section with water, the plasmolytic cells gradually reassume their normal appearance.

From such observations as these it is concluded that the passage of substances in solution into or out of the protoplasm is controlled by the protoplasm itself so long as the cell is living.

If Fern prothalli are not available, the above observations may be made on the epidermis, stripped off from a fresh leaf of *Lilium*. The epidermal cells are large, and clearly nucleated, but contain little or no chlorophyll.

IV. These **osmotic properties** of the cell can be easily studied in cells which have coloured cell-sap, such as those of the garden Beet: this will at the same time serve as a first exercise in cutting sections from a solid mass of tissue.

Cut transverse sections (p. 6) of a piece of a fresh Beet-root.

they must be sufficiently thin to be transparent, but of such thickness that at least some of the cells shall remain uninjured. mount in water : observe—

1. The thin **cell-walls**

2 The layer of **protoplasm** which lines the cell-wall.

3 The red **cell-sap** filling the cavity of the cell (**vacuole**).

Note that the red sap does not escape from uninjured cells.

a. Mount a section in water, and run some 5 per cent. salt solution under the cover-slip ; it will be seen that, as before, water is withdrawn : and the protoplasm contracts round the remaining cell-sap, now more deeply coloured owing to concentration. As in the cells of the Fern prothallus, so also here fine protoplasmic threads run from the contracted protoplasm to the cell-wall. Wash out the salt solution with water, and some at least of the plasmolyzed cells will gradually reassume their original appearance.

b. Examine another section which has been treated with alcohol, and thus killed ; the red sap diffuses out of the cells hence it is evident that though the colouring-matter does not diffuse out of a living cell, it diffuses readily out of a dead cell.

V. In order to observe the **movements of protoplasm** in the living cell, mount in water a "leaf" of *Nitella* : examine it first with a low power, and observe that it is composed of large cells, each limited by a definite cell-wall. Select one cell which lies conveniently for examination under a low power, and focus through the cell-wall upon the protoplasm, so as to see the green chlorophyll-granules embedded in it. Note that they are stationary ; a clear colourless line with no chlorophyll-grains will be seen taking a spiral longitudinal course along the cell : it is known as the **neutral line**. Select a spot in the cell where the neutral line is uppermost, focus carefully upon it with the high power, past the chlorophyll layer, and note the **streaming movement** of the colourless protoplasm within, recognised by the motion of the various bodies borne along by the current. Note further that the movement on one side of the line is towards the apex of the cell, on the other towards its base : the protoplasm on the line between the two streams is motionless, and is the line free from chlorophyll, viz., the **neutral line**.

This type of protoplasmic movement is called **rotation**. More complicated movements are to be seen in various hairs, and notably in those which cover the base of the stamens in species of *Tradescantia*. Remove a few of the hairs from a stamen of an open flower, and mount them in water. Observe under a low power the moniliform hairs, each composed of a row of barrel-shaped cells. Focus the high power upon one of these cells, and note the limiting **cell-wall**, and **protoplasmic streaming** threads or bridges of protoplasm, irregularly disposed, pass from the peripheral protoplasm towards the centrally disposed, spherical **nucleus**.

Examination of these threads will disclose movements of the protoplasm in various directions: these more complicated movements are collectively termed **circulation**. They are, however, essentially similar in nature to the simpler movements of rotation.

The following observations may be made on either of the above specimens. Heat the slide over a spirit-lamp to boiling point: the movements will, on examination, be seen to have stopped, the cell having been killed by the high temperature.

Treat another preparation, in which active movement is going on, with iodine solution: the movement will be arrested, the cell being killed: the protoplasm will be stained brown.

Similar movements are to be seen more or less clearly in living cells generally, and are easily observed in cells of the leaf of *Vallisneria spiralis*, *Elodea canadensis*, *Hydrocharis morsus-ranae*, &c.

III

COMMON MICRO-CHEMICAL REACTIONS

A FEW further practical exercises will now be given, involving the use of common methods and reagents, and leading to a fuller knowledge of the appearance and reactions of the parts of the cell, and of some of the bodies commonly contained in it

I Cell-walls.

A Cellulose Walls.

Take some ordinary unbleached "cotton wool," which consists of unicellular hairs from the surface of the seed of the cotton plant (*Gossypium*). Moisten first with alcohol, and then soak in water

a Mount a small quantity in water, and examine first with a low, and then with a high power: observe—

- 1 The long, filamentous, unicellular hairs, which compose the "cotton wool," coiled irregularly together
- 2 The rather thick, highly-refractive and colourless cell-wall.
- 3 The remains of granular protoplasm, which may still be seen within

b Soak a small quantity of the cotton for a few minutes in iodine solution in a watch-glass, mount in iodine solution, and note the cell-walls stained slightly yellow.

c Mount a small quantity of the cotton which has been thoroughly soaked with iodine, in a single small drop of concentrated sulphuric acid diluted with an equal volume of water the greatest care is to be observed in the use of this reagent,

so that it shall not gain access to the stage, or the objective ; only a very small quantity is to be used, and *the slide should be washed in water directly the observation has been made*. A low power will suffice to show that—

1. The cell-walls swell greatly, and in an irregular form, and ultimately lose their sharp contour.
2. They assume a blue colour. This colouring is often not uniform, and this reaction, though trustworthy as positive evidence of the presence of cellulose where the blue colour is obtained, is not secure as proving the absence of cellulose if the blue colour be not seen

d Mount a fresh piece of the soaked cotton in chloro-zinc-iodine, and observe that the cell-wall stains a more or less distinct blue or a pinkish violet according to circumstances the protoplasm, of which a small quantity may remain in the hairs, stains yellow

e. Mount still another small quantity of the soaked cotton in acid solution of aniline sulphate, and observe that the cell-walls do not stain.

f. One of the most characteristic reactions of cellulose may be observed as follows :—

Prepare an ammoniacal solution of cupric hydrate (see Appendix A) : take, in a pair of forceps, a small quantity of cotton-wool, and immerse it in the fluid. It will be seen that the separate hairs of the cotton lose their identity, coalesce into a gelatinous mass, and are finally dissolved.

The solution, and antecedent swelling of the walls may be observed on a slide under the microscope if a very small quantity of the cotton-wool be mounted in the solution.

These reactions may be repeated on other tissues, e.g. the endosperm of the Date.

B. Lignified Walls.

For the reactions of lignified walls the wood of the Pine will serve : for instance, sections may be cut from an ordinary wooden match. Having cut thin transverse sections, soak them first in alcohol to remove bubbles of air : mount one of them in glycerine, and observe under a high power the very regular network of cell-

walls, which are of almost uniform thickness, and are colourless or slightly yellow protoplasm is practically absent in this tissue.

a. Treat a fresh section with iodine solution, and note that the walls stain distinctly yellow.

b. Mount a section thus thoroughly stained with iodine in a single drop of sulphuric acid no blue colour is produced, the walls swell as do the cellulose walls, but their colour is brownish.

c. Mount a fresh section in chlor-zinc-iodine ; the walls stain yellow, with no trace of blue.

d. Mount another section in acid solution of aniline sulphate : the lignified walls stain yellow.

By means of the above reactions a lignified wall may be distinguished from a cellulose wall.

C. Corky Walls.

Cut thin sections from a piece of common bottle cork : soak them first in alcohol, in order to remove air bubbles, and then in water : mount a thin section in water, or dilute glycerine, and note under a low power the regular arrangement of the tissue, and the thin, pale yellowish or brown cell-walls, with sharp definition and the absence of cell-contents.

a. Treat a section with iodine solution : the walls stain yellow.

b. Treat another section with chlor-zinc-iodine : the walls stain yellow or brown.

c. Treat as above directed (p. 26), with iodine and sulphuric acid : the walls are yellow or brown, and do not swell, but retain their sharp outline.

d. Treat a fresh section with Schulze's macerating fluid (see Appendix A), and warm gently at first : the corky walls turn yellow : then boil vigorously (this should be done at some distance from the microscope, as the fumes given off are apt to attack the metal), and on cooling re-examine : the corky walls, if the reaction be complete, will be found to have lost their definite outline, and to have run together into irregular viscid drops of ceric acid, which is in some measure soluble in the mixture when hot, and is reprecipitated on cooling.

This reaction may with advantage be performed in the bulk, by cutting some shavings of cork, and boiling them for some minutes in Schulze's macerating fluid they will be seen to lose shape, and coalesce into a viscid mass. this is soluble in warm alcohol, benzol, &c.

D. Mucilaginous Walls.

Soak seeds of *Linum* in water for an hour. Observe that the surface of the seed, which was before glossy and hard, is now covered by a pearly layer of transparent mucilage.

Cut thin transverse sections of a dry seed (the razor must be used dry, or wetted with alcohol, or pure glycerine): mount in pure glycerine, and examine the superficial layer of cells of the testa.

Note (1) their thick stratified walls, with a superficial cuticle; (2) the middle lamella, faintly marked by its optical properties.

Dilute the glycerine with water, and note that the thick walls swell slowly, the stratification and middle lamella becoming much more obvious. The swelling is often seen to rupture the cuticle; the swollen mass protrudes, and in this way the transparent layer is formed on soaking the seeds. Though the mucilaginous walls swell with water, they are not dissolved this will be seen on staining. Treat a section with corallin-soda solution: the mucilage stains pink

Treat a fresh section with a watery solution of methylene blue: the mucilage will stain.

II. Protoplasm and Nucleus.

The protoplasm of the cell, and the nucleus, may be observed in the living condition as described in the preceding chapter; but in order to recognize the more minute details, and in order to make permanent preparations of these bodies, more complicated methods of treatment are necessary.

The protoplasm and nucleus must be first fixed and hardened (see above, p. 4): the most convenient hardening agent is absolute alcohol; if picric acid be used it must be very completely washed out from the tissues before staining. For other methods of fixation and hardening, reference may be made to larger works.

Harden the young flowering stem of a common Hyacinth, not more than three or four inches in length, in alcohol: cut longitudinal sections of the basal portion of it, and stain with Kleinenberg's hæmatoxylin (see Appendix A) till the sections are deeply coloured, then wash thoroughly with absolute alcohol in a watch-glass: transfer them (drying off all superfluous alcohol with blotting-paper) to oil of cloves, or turpentine, in which they should be left for some minutes, so that the fluid may thoroughly permeate them: then mount in Canada balsam dissolved in benzol.

Examine sections thus treated under a high power, and observe the chief bulk of the tissue to consist of square or oblong cells of considerable size: the following parts are to be recognized—

1. The **cell-wall**, which is uniformly thin, and is stained.
2. The **protoplasm**, lining it internally, which is also stained.
3. A large central **vacuole**, which is not stained, and is usually traversed by fine bridges of slightly stained granular protoplasm: these suspend it in a central position—
4. The **deeply stained nucleus**: it may be observed in many cases that the nucleus does not occupy a central position, but is embedded in the peripheral protoplasm, while the whole cell-cavity is occupied by a large vacuole.

Pith of a very young shoot of the Elder will also serve as good material for these observations; the young shoot should be treated as above directed, and longitudinal sections will afford similar results. As an alternative method of preparation, which has the advantage of simplicity, stain the sections from material hardened in alcohol, with a solution of methyl green in weak acetic acid, wash with weak acetic acid, and mount in dilute glycerine: the nucleus only is distinctly stained in this case, but the results are as a whole less satisfactory than when the former method is used.

Division of the nucleus, and of the cell.

In sections prepared as above, the cell- and nuclear-division may be seen in progress, and by comparing different stages the history of the process can be constructed. For this purpose it may be found better

to stain with Heidenhain's hæmatoxylin (see Appendix A), which stains especially the **chromatin** of the nuclei ; and mount as before.

Note in the sections, cells in the **quiescent state**, as above described. The nuclei have a definite outline, and are finely granular, with one or more deeply stained **nucleoli** within.

Note further the following chief phases of the process of division :—

(1) Some cells will show a larger nucleus of coarsely granular appearance : in these division is about to take place.

(2) In others the nucleus will have lost the definite outline, and coloured threads of **chromatin** will be clearly marked off from the colourless matrix of **achromatin**.

(3) The chromatin threads, after breaking up into distinct portions, the **chromosomes**, will have taken position about the equator of the slightly spindle-shaped nucleus.

(4) The chromosomes (after division, of which evidence may be seen) arrange themselves in equal numbers towards the **poles of the spindle**.

(5) The chromosomes gather into two masses at the two **poles**, while between are seen the transparent **spindle-threads**.

(6) Granules appear upon the spindle threads at the equatorial plane.

(7) The granules fuse to form the **new cell-wall**, which is extended to the lateral walls of the parent cell.

(8) The parent cell is completely divided, with one reconstituted nucleus in each half, in appearance and position like that of the parent cell.

III. Starch.

a. Scrape the freshly-cut surface of a Potato tuber lightly with a knife, and mount a small quantity of the scrapings in water : examine first with a low, and then under a high power, and observe scattered through the water a large number of somewhat ovoid, colourless, bright-looking, *i.e.* highly refractive bodies : these are **starch-grains** ; near to one end, which is usually slightly pointed, is a round clear spot, the **hilum**. The grain will show a **stratified** structure : the layers of stratification near the hilum are almost circular and concentric ; the more external layers are excentric and elliptical, and are wider on the side further from the hilum ; many of them between the hilum and the broader end of the grain are incomplete ; hence the layers are more numerous between the hilum and the broad end than between the hilum and the pointed end of the grain.

Here and there may be seen **compound grains**, consisting of two small grains in contact by their broad ends, and invested by several layers common to both.

b. Sections should also be cut from the Potato so as to show the starch-grains *in situ* in the cells. The razor should be wetted with water, and one section (the thinnest cut) should be mounted in water. a section which runs out to a thin edge will be found to be best. examine under a high power, and observe—

1. The numerous **starch-grains** as before.
2. The thin **cell-walls** partitioning off the cells which are of considerable size, and each of them may contain a large number of starch-grains.
3. The **protoplasm**, which is so scanty as often to escape observation.

c. Mount a small quantity of starch-grains in water as before, and irrigate with iodine solution. the starch granules will stain a more or less deep blue according to the strength of the solution: this is the characteristic reaction of starch.

d. Treat another preparation of starch with strong chloro-zinc-iodine: the starch-grains will as before assume a blue colour, but they also swell, and lose their bright, high refractive properties. This **fact** is to be borne in mind when treating tissues containing starch with this reagent.

e. Mount a fresh slide of starch in water, and irrigate with solution of potash: observe that as the reagent gains access to the granules they swell, and at the same time assume a dull appearance, their high refractive power being lost as they take up additional water of imbibition under the influence of the reagent. Now wash out the potash thoroughly with water, and irrigate the preparation with iodine solution: the swollen grains will still stain blue, though much paler than before, showing that the swelling with potash does not fundamentally alter the nature of the starch.

f. Mount some fresh starch in water, and heat it over a spirit-lamp till it boils: on examination under the microscope, the grains will be seen to have swollen and lost their high refractive power, forming **starch-paste**: staining with iodine will

produce the blue colour, and show that they are only swollen, not dissolved : compare the effect of potash. A temperature of about 65° C. is sufficient to cause this swelling.

g. Digest starch-grains in saliva for some hours at a temperature of about $45-55^{\circ}$ C. Examine them subsequently under the microscope they will be found to have lost their high refractive power. Stain with iodine they give a pale blue or yellowish colour.

IV. **Chloroplasts**, or **chlorophyll-corpuscles**.

Mount a fresh Fern prothallus in water, and note as in the last chapter the several parts of the cells which compose it, and especially the green **chlorophyll-corpuscles**, which are usually of discoid form, sharply defined from the surrounding colourless protoplasm.

Observe here and there granules of oval or biscuit-shaped outline : these are stages in the process of **division**, by which means the chlorophyll-corpuscles increase in number. Drawings of a series of such forms should be made so as to illustrate the process of division.

Treat with alcohol ; the green colouring substance (**chlorophyll**) will be seen to be dissolved out of the granules, but they will retain the same definite outline as before.

A solution of chlorophyll may be prepared in bulk by taking a quantity of fresh grass or healthy cabbage leaves boil them in water, dry by pressure, and spread them out in a dark place ; when completely air-dry soak them in alcohol in a dark place ; pour off the alcohol and filter. It will be deeply coloured, owing to the solution of the chlorophyll, while the mass of leaves will be bleached.

Take a small quantity of the solution in a test tube, and examine it first by light transmitted through it—it will appear deep green in colour. Examine it now by reflected light . the solution will appear a deep dull red. The solution is thus *dichroic*.

The various other bodies, which are found either having definite form (such as aleurone grains), or in solution in the

cell-sap (such as inulin), will be described as opportunity offers, later in the book. a special section at the end of the description of the Angiosperms will be devoted to the study of the nutritive materials stored in seeds and fruits. The reactions by which the common components of the plant-body may be recognized, are stated concisely in Appendix B at the end of the book.

Remarks on Staining, Clearing, and Permanent Mounting.

Staining.—It is often useful to stain sections in order to bring out certain points in their structure, or to distinguish between bodies of nearly the same refractive index and appearance, but of different nature. A very large number of colouring matters have been used for this purpose, some of which are mentioned in Appendix A. a very few of them will suffice for ordinary laboratory work, and *none are ever to be used without a definite purpose.*

Staining is best performed by placing some of the staining fluid in a watch-glass, and immersing the sections in it. The exact strength of the fluid, and the time of exposure of the sections to its action varies in each case, and must be ascertained by preliminary trials. As a rule, when differentiated staining is desired (see Safranin, Append. A), the best results are obtained by using a dilute solution, and by exposing the sections for a long time to its action; after staining and before mounting for observation, it is as a rule necessary to wash the sections in order to remove the superfluous staining fluid; when the staining substance is dissolved in alcohol, the sections are to be washed out with alcohol; when dissolved in water they are to be washed with water: in the case of iodine staining this need not be done, as these colourings fade rapidly when the staining fluid is removed.

Clearing the Preparations.—If it is not desired to observe the details of structure of the protoplasm or of the nucleus, the best clearing agent for ordinary use is a solution of potash, either in water or alcohol.

The clearing action of potash is due to the swelling of various

parts of the cells and their contents, so that they become more transparent ; at the same time it dissolves many of the granules in the protoplasm, and saponifies the oil-drops. The swelling caused by the action of the solution in water is often too great, especially when it is desired to see the cell-walls distinctly ; this difficulty may be got over by the use of the alcoholic solution.

After treatment with the aqueous solution of potash, the sections should be washed in distilled water, and after treatment with the alcoholic solution in dilute alcohol ; the sections, in either case, may be mounted in glycerine : or the sections may be treated at once with a mixture of potash solution and glycerine, but in any case the potash must be washed out before mounting as a permanent object.

If treatment with potash solution does not readily make the tissues transparent, the action of the reagent may be accelerated and intensified by warming over a spirit-lamp. If the action be too strong, and the tissues become too transparent, this may be corrected by neutralizing with acetic acid.

Another method which gives good results, especially in clearing growing-points, is by the use of "eau de javelle" (see Appendix A). The object, either fresh, or after hardening in alcohol or picric acid, is mounted under a cover-slip in "eau de javelle" for three or four, to ten or fifteen minutes, according to the rapidity of action of the reagent ; very gentle warming over a spirit-lamp will quicken the action. It is then to be carefully washed with water, next with dilute acetic acid, and it may finally be mounted in glycerine.

All the above methods involve the *partial or complete disorganization of the protoplasmic body* : the following method of treatment has the advantage of preserving the structure of the protoplasm and of the nucleus, and it is specially applicable to material in which the protoplasm has been fixed by alcohol, or by picric acid and alcohol. The sections (after staining, if that is considered necessary) should be placed for a few minutes in absolute alcohol ; they should then be transferred to a watch-glass, containing either a mixture of turpentine and creosote (four parts of the former to one of the latter), or some oil of

cloves ; sections which have been stained with aniline dyes are best cleared by cedar-wood oil ; they should be left in the clearing agent for a short time, until they appear to be quite transparent, and should then be mounted in a drop of Canada balsam or Dammar.

Permanent Mounting.—It was pointed out in the previous chapter that objects mounted in water cannot easily be kept, while the objects do not appear so transparent in water as in some medium of higher refractive index. The media most commonly used are glycerine, glycerine jelly, Canada balsam, and Dammar.

Glycerine.—This may be used for objects prepared from fresh material, or hardened with alcohol, &c., and is especially suited to objects stained with ammoniacal solution of hæmatoxylin, carmine, and many of the aniline colours ; it is also used for objects cleared by potash, or “eau de javelle.” Dilute glycerine should be used for this purpose, consisting of a mixture of pure glycerine with an equal bulk of water.

In order to make the preparations mounted in glycerine permanent, the cover-slip should be fixed to the slide by applying a coating of gold size, Brunswick black, or Canada balsam dissolved in benzol, round its edge with a brush. If a circular cover-slip be used, a turn-table will be found to save much time in this process. Care should be taken that no glycerine is on the slide outside the cover-slip ; if any is there it should be carefully cleaned off before applying the varnish.

Glycerine Jelly.—Objects which may be mounted in glycerine may equally well be mounted in glycerine jelly, in which case since the jelly sets firmly, it is unnecessary to use any cement or varnish. The sections should be previously soaked for one or two days in glycerine so as to remove water or alcohol from them. A trace of carbolic acid should be added to the glycerine jelly in order to prevent the growth of Fungi.

Canada Balsam.—This is a highly refractive medium, and is thus well adapted for lending transparency to objects. It is specially suited to sections stained with hæmatoxylin. Water must be completely extracted from the objects before mounting, by treatment with absolute alcohol, or strong methylated spirit ;

they are then to be transferred to oil of cloves, or a mixture of turpentine and creosote, or cedar wood oil, and finally mounted in Balsam

The stain produced by aniline colours is apt to fade, so that they are not to be recommended for preparations which are to be kept for a long time. The staining of hematoxylin also fades, but more slowly. In order to prevent fading, the preparations should be kept in the dark.

PHANEROGAMÆ

ANGIOSPERMS.

MATURE SEED AND EMBRYO.

(A) *Dicotyledons*

I. (a) Soak some Broad Beans for 24 hours in water : selecting one which is fully swollen, note its flattened form, and the dark blotch (the **hilum**) at one edge of it : this is the base of the seed, by which it was attached to the parent plant. Dry the surface of the seed and squeeze it gently, water will be seen to exude from a small hole close to the hilum. This hole is the **micropyle**, and is a guide to the position of the technical apex of the seed, the whole being of the curved or campylotropous type.

Remove the tough outer **seed-coat**, derived from the integuments, and the bulky, yellowish **embryo**, which occupies the whole space within the seed-coat, will then be disclosed. It is to be observed that there is here no tissue derived from the nucellus, and no endosperm, and the seed is therefore described as **exalbuminous** : note the following parts of it.—

1. The two fleshy **cotyledons**, which are attached at their base.

2. The conical **radicle**, which lies externally, and in the seed has its pointed apex directed towards the micropyle.

3. Separate the two cotyledons, and between them observe a bud, the **plumule**, composed of numerous small plumular leaves.

(b) Compare with the Bean the flattened seed of the Cucumber or Gourd the micropyle may be found by the same means as before, at the pointed end of the seed, and close to it is the small scar of the hilum this seed is of the anatropous type and is **exalbuminous**. Peel off the leathery seed-coat, and note the parts of the straight embryo, viz —

1. The radicle directed towards the micropyle.
2. The two cotyledons ; fleshy as before.
- 3 Between them the very small plumule.

(c) Examine fruits of the Sycamore (*Acer pseudo-platanus*); before falling the fruit divides into usually two **mericarps**, each of which is a winged **samara**, containing a single seed Remove the dry **pericarp**, and examine the seed It is almost spherical, the prominent ridge marks the position of the radicle The scar at its end is the **hilum**, or point of attachment

Remove the brown skin, or **testa** ; and note the following parts of the green fleshy embryo —

1. The two pleated **cotyledons**, closely folded.
2. The cylindrical **radicle**.
3. Separate the cotyledons, and the **plumule** will be seen, as a small bud, at the base of the groove between them.

It is an **exalbuminous** seed.

(d) Compare the structure of the **albuminous** seed of the Castor oil (*Ricinus communis*), observing externally the hard, bright, variously marked **seed-coat**, which has attached to it at the basal end a wart-like swelling—the **caruncle**.

Remove the seed-coat, which is brittle and easily cracked ; note beneath this a thin papery white layer this closely invests the white oily mass of the **endosperm**, a tissue which is not present at the period of maturity in any of the seeds above described. Cut this through transversely and a flattened central cavity will be found, lined on either side by one of the thin flattened **cotyledons** of the embryo. Lay open the endosperm of another seed longitudinally, by a cut following the plane of the flattened cavity it will then be clearly seen that the straight embryo is embedded in a mass of endosperm, and that it consists of two cotyledons, radicle, and plumule.

(e) Examine the ripe fruit of the Sunflower (*Helianthus annuus*). The "seeds" sold for sowing are really fruits (achænia), including the products of development of both ovary and ovule : each is a dry inferior **achæmium**, with narrower basal, and broader apical end : at the latter is a scar, where were inserted the style and other floral organs.

Compare fruits *in situ* on the floral receptacle.

Dissect off the brittle **pericarp**, from the anatropous and exalbuminous seed, which it incloses.

Note the delicate **seed-coat**, and, within this, the straight **embryo**, of which the **radicle** is directed towards the micropyle (*i.e.* towards the base of the fruit), and the two **cotyledons** towards the apex of the fruit.

Separate the two cotyledons, and note between them, at their point of attachment together, the minute **plumule**.

(f) Examine the ripe fruit of *Heracleum giganteum* : it is a **schizocarp**, which splits when ripe into two halves (**mericarps**), each of which is marked by four **glandular vittæ** on its outer, and two on its inner surface.

Note at the apex of the fruit the two **stigmas**, which still persist. Below these the ring-like **scar** of insertion of the other floral organs : the ovary is thus **inferior**. Separate one flattened mericarp from its attachment to the thread-like carpophore : open it carefully from the base and dissect out the **single seed**, which is pendulous from the top of the cavity. Cut median longitudinal sections from the seed, with a razor. mount in water, and note with a lens, or low power—

1. The thin peripheral **testa**.
2. The **endosperm**, a bulky mass of nutritive tissue.
3. The **embryo**, embedded in the endosperm, and attached at the micropylar end, near the funiculus or stalk of the ovule.

The whole is an **inferior fruit**, with one **albuminous seed** in each mericarp.

(B) *Monocotyledons.*

(a) Soak fruits of the Maize (*Zea Mays*) in water for several hours. The fruit is a caryopsis, and results from the development of both ovule and ovary ; its form is compressed conical,

the apex of the cone being the basal point of attachment of the fruit.

Strip off the external coat of the fruit. this represents both the **wall of the ovary** and the **integument** of the ovule.

Distinguish in the body of the fruit which remains—

1. A lateral, smaller, white portion this is the **embryo**.
2. A larger yellow part, which forms the greater mass of the fruit : this is the **endosperm**.

Separate the embryo from the rest, and note its shape.

Cut longitudinal sections of the dry fruit so as to traverse the embryo in a median plane : clear in potash, mount in glycerine, and examine with a low power. observe.—

1. The coat of the fruit, consisting of two layers, the **pericarp**, and **seed-coat** or **testa**. Note at the apex of the fruit the remnant of the **style**, and the scar of attachment at the base.

2. The **endosperm**, consisting of thin-walled parenchyma.

3. The **embryo**, which is in close apposition to the endosperm : the part which is in contact with it is the **scutellum** ; it extends over the whole surface of contact with the endosperm, and almost completely surrounds the body of the embryo itself.

The body of the embryo consists of—

4. An **apical bud**, with several sheathing leaves, which surround the apical cone.

- 5 The **radicle**. Outside the radicle, and continuous with the root-cap, is a root-sheath, or **coleorhiza**.

(b) Compare a grain of wheat (*Triticum vulgare*) . it is oval, with one side deeply grooved, the other smooth and convex, with a depressed area at the base. this marks the position of the **embryo**. Cut median longitudinal sections, so as to traverse the embryo. Mount in water and examine with a lens or a low power. Observe :—

1. The brown band of **pericarp and testa**.
2. The mass of white floury **endosperm**.
3. The **embryo**, with parts similar to those above noted for the Maize.

(c) Examine seeds of the common Onion (*Allium Cepa*), they are black, and of irregular conical-prismatic form, the apex of the cone, *i.e.* the micropylar end, is the **base of attachment**.

After soaking the seeds in water so as to soften them, cut one of them longitudinally with a razor, following the plane in which the seed is slightly flattened ; the section will probably show—

1. The **testa**, a complete external coat.
2. The **embryo**, a curved body, of which one end—the **radicle**—points towards the base of the seed, while the other more sharply-curved end is the **single cotyledon**.
3. The **endosperm**, a more transparent tissue, which fills up the remaining space.

(d) Examine the “stone,” that is, the **seed**, of the Date Palm (*Phoenix dactylifera*) note —

1. The thin brown peripheral film of the **testa**.
2. The hard stony mass of semi-transparent **endosperm**, with an irregular groove down one side.
3. The **embryo**, which will be found as a small soft body, about the middle of the smooth convex surface of the “stone” ; its position is readily seen by a shallow depression, into which a needle can easily be driven.

The above specimens are all albuminous seeds of Monocotyledons.

GERMINATION.

(A) *Dicotyledons*.

(a) Examine seedlings of *Helianthus* which have been germinating for different periods from one day to one week, and observe the following points in the process of germination :—

1. The internal parts of the fruit swell, and cause the brittle pericarp to split longitudinally.
2. The radicle protrudes, and curves downwards.
3. The hypocotyledonary stem elongates, so that the pericarp and seed-coat are carried upwards by the cotyledons, which remain inclosed by them for a considerable time.
4. The coats of the fruit fall from the cotyledons, which soon turn green, and expand as assimilating leaves, with the plumule seated between them.
5. The plumule develops leaves, which expand in succession.

6 The radicle has meanwhile elongated, and produced lateral roots, in acropetal succession

Notice that when the young root is removed from the soil many particles adhere to it, especially at some distance from the apex—these are held by the **root-hairs**, which attach themselves closely to the particles of soil.

(b) Compare the germination of the Sycamore: the pericarp and testa split; the radicle curves downwards, and the cotyledons, remaining for some time enclosed in their coverings, are raised above ground by the growth of the hypocotyl; ultimately they free themselves, and unfold as green assimilating leaves.

(c) In the Cucumber, the germination of the seed is of the same type as the above; but note that the seed-coat is retained below ground. This is due to the outgrowth of a spur at the base of the hypocotyl—by means of root-hairs on the under side of the spur, the seed-coat is held down, while the cotyledons are withdrawn

(d) With the above compare seedlings of *Rumex* in various stages of germination: in the main features the results are the same, but note especially that the endosperm remains for a long period in close connection with the cotyledons, and that as the seedling grows that tissue loses its firmness and density, owing to the abstraction of the nutritive substances stored in it, and their transfer through the cotyledons to the seedling.

The above examples of germination are styled **epigeal**, since, owing to the strong intercalary growth below the cotyledons, these are raised above the level of the soil. The cotyledons themselves are freed from the seed-coat chiefly by their own growth in length—but in the Cucumber by the help of the spur.

As an example of **hypogeal** germination, examine seedlings of the Broad Bean or Pea. Observe that the intercalary growth here is chiefly in the axis *above* the cotyledons: the result is that the seed-coat and cotyledons remain below ground, while the products of the plumule rise above it. Examine the root system, and note the main root, bearing an acropetal sequence of lateral roots: and that the latter are arranged in longitudinal rows usually four in number.

Similar observations may be made on the germinating Acorn.

(B) *Monocotyledons.*

(a) Comparing plants of Maize which have been germinating for different periods, the following facts in the history of germination may be observed :—

1. The fruit swells.
2. The outer coat ruptures opposite the apex of the radicle, which soon protrudes, bursting through the coleorhiza also, which appears as an irregular sheath round the base of the young root. Since the coleorhiza is thus burst through by the young root, it is clear that the epidermis of the shoot is not continuous with the piliferous layer of the root.
3. The rupture of the coat extends upwards to the point opposite the apical bud, which also emerges.
4. The root elongates, and forms lateral roots. Other lateral roots (usually two) burst out above the insertion of the scutellum. These soon equal the primary root in length, hence there is no well marked tap-root.
5. Leaves of the plumule unfold, and gradually turn green: the leaf inserted lowest, which was the outermost of those composing the plumule, remains small and develops no expanded lamina. This is the **cotyledon**, according to Hofmeister and other writers.

b. The above is a hypogean germination of an albuminous seed. In order to observe the storage material in the endosperm, and the relation of the scutellum to it during germination, cut longitudinal sections from a young seedling, with leaves about three inches long, so as to traverse the whole fruit and the contiguous part of the seedling in a median plane: mount in water, and irrigate with a solution of iodine. Observe—

1. The large quantity of starch in the cells of the endosperm.
2. That in the neighbourhood of the surface of the scutellum the starch-grains are in course of demolition, and that the central part of each is first attacked.
3. That the surface of the scutellum facing the endosperm is covered by a densely protoplasmic **epithelium**, and that no starch-grains are present in it.

c. In the seedling of the wheat a similar arrangement of parts may be seen to that described for the Maize: both are hypogean.

d. Compare the germination of the Onion. The root first emerges from the seed, and grows downwards, the cotyledon elongates, and forms an arch above ground, while the albuminous seed in which its apex is inserted is later carried above ground, when the cotyledon straightens itself. The plumule is seated at the base of the cotyledon, and is surrounded by it.

Similar observations may be made on the germination of the Date.

ANATOMY OF THE VEGETATIVE ORGANS

DICOTYLEDONS

Observations with the Naked Eye

1. Some seeds of the Sunflower should be germinated in a pan, and the young seedlings, after forming a few leaves, should be bedded out, and allowed to grow for about three months. Examine a well-grown specimen of that age, as a whole. The main axis or **stem** is stout, herbaceous, and erect. It often develops to a considerable length without branching. It is cylindrical, slightly striated below, while the higher parts of it, where the lateral branches are developed, are polygonal. Its surface is studded by stiff hairs, which are especially obvious on the lower portions of the internodes.

The stem bears laterally numerous **leaves**, which are simple, petiolate, cordate-acuminate, the margin slightly serrate, venation palmate-reticulate, the surface hirsute. The arrangement of the leaves at the lower part of the plant (and including the **cotyledons**, which wither at an early stage) is opposite, or in whorls of three; higher up, this arrangement merges gradually into the alternate.

The stem is terminated by a **bud**, which may consist only of closely aggregated **foliage leaves**, or it may enclose the **reproductive organs**, which are contained in numerous flowers, closely aggregated so as to form a characteristic **inflorescence**.

—the **capitulum**, or **head**. Similar buds, in earlier stages of development, may be observed in the axils of the leaves (**axillary buds**).

Wash the roots and examine them. They are fibrous, and branch profusely. The primary (tap) root, and earlier developed lateral roots are thicker than the later developed roots of a higher order, the latter being successively thinner. This is due to the fact that the roots undergo a process of secondary thickening.

II. Cut the stem of a well-grown plant transversely at its thickest part, and smooth the surface with a razor.

The most prominent object in the section will be the massive, white, spongy **pith**, which occupies the centre.

Around this will be seen, arranged more or less regularly in a circle, and near the periphery, a series of more solid-looking masses of tissue; these are the **vascular bundles**, which constitute the conducting tissue.

III. In order to obtain a clear idea of the course of these bundles along the stem, and of their connection with those of the leaves, cut off a piece of the stem, so as to include the insertion of a leaf or **node**, and about two or three inches of stem above and below that point. Bisect this longitudinally in a plane perpendicular to the median plane of the leaf, *i.e.*, so that one of the pieces will bear the whole base of the leaf. Clear away the pith with some blunt instrument, taking care not to injure the vascular bundles. This process will be made easier if the stem be previously boiled in water for about ten minutes.

Now dissect out carefully the course of the several vascular bundles, clearing away as much of the internal parenchyma as possible. Treat the whole preparation with acid solution of aniline sulphate for about five or ten minutes. The vascular bundles will be stained **yellow**, and their course directly up and down the stem will be easily followed. It will be apparent that in the internodes the bundles run **parallel to one another**, and as a rule without lateral fusion.

IV. Dissect carefully the region of insertion of a leaf, and note that certain of the vascular bundles—usually three of

them—pass out from the stem into the leaf-base: if their course be pursued further up the leaf, they will be found to branch. If a sufficiently long piece of the stem be dissected it would be found that all the vascular bundles of the stem are similarly **leaf-trace bundles**. Observe that lateral fusions between the bundles occur occasionally in the internodes, but are **more** frequent at the nodes. If a single leaf-trace bundle be followed from the leaf downwards, it will be found that its course ends in such a fusion.

Microscopic Observations.

*** Young Stem.**

The material should be kept in spirit for some time, to remove resin and air, and to harden the tissues; but this is not indispensable, and fresh material may be used, though it is not so satisfactory.

1. Cut transverse sections of a young stem of the sunflower, about one-eighth of an inch in diameter.

If the sections be cut from the hypocotyledonary stem, though they will correspond in all important points to the following description, they will differ in some minor details; thus hairs will be absent.

Mount some in glycerine, others in chlor-zinc-iodine. Observe successively, passing from the periphery, under a low power:—

1. The **epidermis**, a single peripheral layer of cells, completely covering the surface, but not very well defined from the underlying tissues. The margin is not perfectly regular, but is here and there extended outwards at the regions surrounding the bases of the large multicellular hairs, which may be recognised as being products of the epidermis.

2. Within this lies the **cortex**. Its internal limit may be seen, in the sections in chlor-zinc-iodine, to be the endodermis, a layer of cells containing starch-grains, which are stained dark blue. This cortex is composed of:—

a. **Collenchyma**, a band of tissue several layers in width, directly below the epidermis. The walls of its cells

are more or less thickened at the angles, where three or more cells meet, the cell-cavity being thus made oval, or circular in transverse section. These are the chief characteristics of collenchyma, of which this is a good type.

b. **Thin-walled parenchyma**, consisting of rounded cells, with obvious **intercellular spaces** : these are also well seen in the collenchyma. **Resin passages** are dotted here and there in the cortical tissues.

c. **Endodermis**, a continuous wavy layer of barrel-shaped cells, in close lateral contact with one another : note the starch-grains in its cells, and for further details see below, p. 50.

3. The **Stele**, or central cylinder, which occupies the whole central region of the stem. Of this, the most prominent structural features are :—

a. The **vascular bundles**, disposed in a ring.

Each vascular bundle consists of two regions :—

i. The inner thicker-walled tissues of the **xylem** or **wood**, yellowish with chlor-zinc-iodine.

ii. A more delicate smaller-celled tissue, the **phloem**, lying next its outer limit. The walls of this tissue are stained blue with chlor-zinc-iodine. The well-marked outer mass, opposite each bundle belongs to :—

b. The **vascular conjunctive tissues**. These are composed of :—

iii. The **pith**, a massive tissue occupying the centre of the stele.

iv. The **medullary rays**, separating the vascular bundles from each other laterally.

v. The **pericycle**, lying between the vascular bundles, and the endodermis, which curves slightly towards the centre of the stem in the spaces between the bundles.

N.B. In **Helianthus** the pericycle opposite the vascular bundles widens out into a mass of tissue, which becomes thick-walled with age—the **sclerenchyma** : while opposite the medullary rays the pericycle is narrower, and thin-walled.

** The Mature Stem.

I. Cut thin transverse sections of a stem of a well grown plant of *Helianthus*, i.e. of a stem more than half an inch at least in diameter

Mount some of these in glycerine or glycerine jelly (these may be kept as permanent specimens), and others in chlor-zinc-iodine. Examine these first with a low power, and observe the general arrangement of tissues as in the young stem above described. The **pith** is much more bulky, and frequently fistular; the **vascular bundles** are larger, and their tissues more definite; while, if the stem is an old one, the bundles may be connected laterally by bands of secondary vascular tissue, thus forming a complete ring. The **pericycle masses** opposite the larger vascular bundles are now strongly **sclerenchymatous**. The **cortex** and **epidermis** are little altered, but appear to have been stretched by the increase of the tissues within.

II. Choose out the thinnest of the sections, and examine it with a higher power, starting as before from the periphery of the stem.

1. The **epidermal layer** will be seen to consist of cells contiguous with one another, without intercellular spaces, but with occasional stomata: the structure of these will be studied in detail elsewhere. The walls, and especially the external and internal walls, are thick, highly refractive, and show a stratified structure. In chlor-zinc-iodine they show the characteristic blue of cellulose with the exception of the outermost layer—the **cuticle**: this is a continuous well-defined layer, which stains yellow, and may thus be easily recognized (see p. 28, reactions of corky walls).

The granular protoplasmic contents of these cells (brown, with chlor-zinc-iodine) are not plentiful, but form a thin layer lining the somewhat rounded cell-cavity. **Chlorophyll-grains** are to be found in these cells: this point is to be noted, since in the stems of many plants chlorophyll is absent from the epidermal cells.

The cells surrounding the bases of the hairs, as well as the

underlying tissue, show a luxuriant growth : in fact the hairs are each seated at the apex of an **emergence**.

2. The **cortex** consisting of :—

a. The **collenchyma**, in which the protoplasmic body resembles that of the epidermis : chlorophyll-grains are numerous. The cell-walls are highly refractive, and stain blue with chlor-zinc-iodine (cellulose) ; they are specially thickened at the angles, where three or more cells meet : in the thickened masses the **lines of stratification** are well seen. Note that the collenchyma is not continuous below the stomata ; also that there is no sharp internal limit to the collenchyma, but it merges gradually into—

b. The thin-walled **cortical parenchyma**, which differs from the preceding in the thinness of its walls, its less copious cell-contents, the larger size of the cell-cavity. **Intercellular spaces**, which result from the splitting of the cell-walls at the points where three or more walls meet, are found in this tissue, and also in the collenchyma ; in the living state they are filled with air, and even in specimens which have been treated with alcohol, air-bubbles may still be found entangled in them.

Observe carefully the **resin-passages**, which occur in the cortical parenchyma. The resin, being soluble in alcohol, has been removed. They are **intercellular spaces**, formed by the splitting of cell-walls. The cavity thus formed is surrounded by small, thin-walled **secretory epithelium**, the cells of which have dense protoplasm and prominent nuclei : they divide both radially and tangentially as regards the passage.

Note that in the epidermis, collenchyma, and thin-walled parenchyma of the cortex, there occur divisions of the cells in a radial direction. Compare the girth of the stem at the upper with that at the lower part of the plant, or that of a young plant with that of an old one. The conclusion will naturally be drawn that the stem increases in girth as it grows older, and since the outer tissues neither peel off, nor do the individual cells increase greatly in width, longitudinal **radial divisions** of the cells are the only alternative.

The innermost layer of the cortex, bordering on the stele, is the **endodermis** : this layer, which is easily identified by its

DICOTYLEDON—STEM

starch in the young stem (p. 48), is less conspicuous in the old stem. It is most easily recognized opposite the vascular bundles, where it borders the sclerenchyma externally. A characteristic feature of the endodermis is a dark dot on the radial walls, which is, however, better seen in other examples (see pp. 64, 65). The cell-walls are faintly yellow with chlor-zinc-iodine (cutinised), and frequently pressed out of shape.

3. All the tissues lying within the endodermis constitute the **stele**. its several component tissues will be examined separately, beginning with :—

a. The **vascular bundles** : but it is not to be assumed that the vascular bundles are sharply delimited from the surrounding conjunctive tissue. Select one of the largest of these for detailed study, beginning with :—

1. The **xylem**, which is next the pith. It consists of elements of various structure : of these the most noticeable are the **vessels**, easily recognized by their large cavity : they are arranged in radial rows, the individual vessels usually increasing in size from the central limit of the bundle. The walls are thick and lignified (yellow with chlor-zinc-iodine, or with acidulated aniline sulphate, see p. 28) ; they have no protoplasmic contents, their further distinctive characters can only be seen in longitudinal sections. **Thylones** may be observed (see below, p. 56), especially in more central vessels. The vessels next the pith are the smallest, and have been crushed and partially disintegrated : they are the first-formed elements of the wood, or **protoxylem** : the rest of the primary xylem is formed later, in centrifugal succession, and is styled the **metaxylem**. The vessels are embedded in tissue composed of two tissue-forms, which, however, are not readily distinguishable in transverse sections : they are the **Xylem-**, or **wood-fibres**, which appear irregular and polygonal in transverse section, and have thick lignified walls : cell-contents are not prominent, or they may be entirely absent :—and the **Xylem-parenchyma**, cells which retain their protoplasmic contents ; their cell-walls are lignified, or of cellulose : the latter is the case with those cells which surround the more central vessels.

ii. The **phloem**, lying outwards from the xylem, consists of

elements of very different structure and function : these are **sieve-tubes**, which appear in transverse section as the larger cavities of the phloem : their walls are rather thin and consist of cellulose (blue, chlor-zinc-iodine). Occasionally these cavities will be found traversed by transverse septa, having a punctuate appearance : they are transverse **sieve-plates** : they are readily detected by the dark staining of the contents surrounding them, with chlor-zinc-iodine. Abutting directly on the sieve-tubes, and appearing as though they had been cut off from the sieve-tube by a longitudinal wall, may be seen smaller cells : these are the **companion-cells**, distinguished by their denser contents. The remaining elements resemble the sieve-tubes in transverse section except in their smaller size, and absence of sieve-plates : these are the **phloem-parenchyma**.

N.B. —Outwards from the phloem lies the thick-walled mass of **sclerenchyma** : this belongs to the conjunctive tissue, and will be described under that head.

Between the xylem and the phloem lies a band of thin-walled tissue, which has not the distinctive characters of either. Its cells show evidence of cell-division, and are arranged in regular radial rows. This active formative layer is the **cambium** : by means of it new tissue may be added both to the xylem internally, and to the phloem externally. Note how in passing from the cambium to the xylem or phloem, the different tissue-elements are differentiated from the originally uniform cells of the cambium. For additional details see the description for *Ricinus* (p. 60), where its structure is more easily observed.

b. The **vascular conjunctive tissues** consist of—

i. A central **pith**, composed of cells which have for the most part lost their cell-contents : they have very thin walls : the walls are slightly pitted, and intercellular spaces small. The cell-cavity is usually filled with air, which replaces the protoplasm ; hence the whiteness of the pith.

ii. **Medullary rays**, here broad parenchymatous tracts, continuous with the pith, and lying between the vascular bundles. They are crossed by lateral continuations of the cambium (the interfascicular cambium) which may already have formed small intermediate vascular bundles.

iii. That part of the conjunctive tissue which lies immediately within the endodermis is the **pericycle**: in this stem, opposite the vascular bundles, it takes the form of **sclerenchyma**, disposed as half-moon shaped masses of tissue, consisting of elements with rounded cavity, in which may be recognised the remnants of protoplasmic contents. The walls are thick, and **lignified** (yellow with acidulated aniline sulphate, or with chlor-zinc-iodine, see p. 28). They also show differentiation into layers, of which the most prominent is the bright-looking **middle lamella**. Perpendicular to the internal surface of the walls **pits** may be seen. The remainder of the pericycle, opposite the medullary rays, remains thin-walled.

III. Cut radial longitudinal sections of an old stem of *Helianthus*, and choosing such as have passed through a vascular bundle (easily recognized with the naked eye), treat them as above.

Bear in mind the observations already made on the transverse sections, and compare those results with the observations about to be made.

Note successively the tissues already observed in the transverse sections. It is not always possible to see all the tissues satisfactorily represented in a single radial section, therefore the study of the tissues and of their relative positions should be conducted by comparison of a number of sections one with another.

1. The **epidermis**, consisting of oblong cells, whose walls and contents present the appearance already observed in the transverse sections. Note the disturbance of their normal arrangement around the bases of the larger **hairs**.

2. Beneath the epidermis lies the **cortex**, composed of:—

a. The **collenchyma**, consisting of oblong cells with thick longitudinal cellulose walls (blue, chlor-zinc-iodine), and thin transverse ends: the contents are protoplasm, with a **nucleus** and **chlorophyll-grains**. Below each of the larger hairs the collenchyma gives place to short thin-walled parenchyma, which, together with the epidermis covering it, forms those **emergences** on the summit of which the hair is seated. Within this is—

b. Thin-walled **parenchyma**, the cells of which are shorter, but wider, than those of the collenchyma ; there is, however, no sharp limit between them : observe transitional forms. The cell-contents resemble those of (*a*), but there is less chlorophyll.

Note the **resin-passages**, the course of which is directly longitudinal : they therefore appear as longitudinal bands of small, oblong, thin-walled cells (**epithelium**).

c. The **endodermis** may occasionally be recognized as the layer of cells immediately outside the stele. Very commonly starch-grains may be detected in its cells.

3. The **stele**.—Supposing the section to have been approximately median through a vascular bundle, the following components will be found to be included in it :—

i. The **pericyclic sclerenchyma**, which appears in longitudinal section as long **prosenchymatous** cells, occasionally divided by more or less oblique septa. The walls are thick, lignified (yellow with chlor-zinc-iodine, or with acidulated aniline sulphate), and pitted : remnants of the protoplasmic contents may be found, especially if the stem cut be not very old.

ii. The **phloem**, consisting of tissues with cellulose walls, (blue with chlor-zinc-iodine), and abundant protoplasmic contents : its several constituents are :—

a. **Sieve-tubes**, long tubes with thin walls and transverse or oblique septa (**sieve-plates**), the structure of which is the chief characteristic of the sieve-tubes ; they are readily recognized in sections treated with chlor-zinc-iodine (or iodine solution), by the deep brown colouration of the contents, which are collected round the sieve-plates.

Treat some sections with potash : the contents and the mass of **callus** surrounding the sieve-plates, swell, and the perforated or sieve-like character of the septum, which does not swell, is then easily recognized. The sieve-tubes will be more easily recognized in sections which have been coloured with cosin (see Appendix A), which stains the contents of the tubes deeply.

A more detailed study of sieve-tubes, and their structure and contents will be given below in a special section. (See p. 62.)

b. Side by side with the sieve-tubes may be found the **companion-cells**, which are smaller sister-cells of the segments of the sieve-tubes, cut off during development.

c. **Phloem-parenchyma**, consisting of oblong parenchymatous cells, with thin, indistinctly-pitted, cellulose walls, and protoplasmic contents.

iii. The **cambium**, a narrow band of oblong cells with very thin walls, and protoplasmic contents. As the tissue in this case differs in no essential point from that in other plants treated elsewhere, and as it is here difficult to study, its description will be deferred, though its presence here must not be forgotten. (See below, p. 60.)

iv. The **xylem**, consisting of :—

a. **Vessels**, which are its most prominent constituents : they are elements with lignified walls, which are variously marked : they have no protoplasmic contents, their wide cavity containing during life, water or gases. The cavity is continuous, owing to the partial or complete absorption of the transverse or oblique septa. Note instances of this partial or complete absorption. According to the various markings, or thickenings of their walls, the vessels may be grouped under the following heads, the first named being the nearest to the periphery of the stem :—

α. **Pitted vessels**, which are the largest, having very wide cavity : their walls are marked with **pits** which appear oval in surface view ; and which have the same characters as the round bordered pits of *Pinus*. (See below.)

Having observed the pits in surface view, focus so as to obtain a longitudinal optical section of one of the walls ; or better, find a place where the preparation is so thin as to show this in real section. Compare this with what was seen in surface view.

β. **Spiral vessels** found in the part of the xylem nearer the pith, those nearest the pith having the spirals less closely coiled. Note transitional forms (irregularly **reticulated**) between spiral and pitted vessels.

γ. **Annular vessels** found at the part of the xylem directly adjoining the pith : the thickening is here in the form of **ribs** ;

in mature stems these vessels are usually more or less disorganized.

The spiral and annular vessels are those formed first in the young stem : and in plants generally these markings are typical of the **protoxylem**.

b. **Fibrous elements**, which are long and pointed : it is difficult to follow one individual fibre throughout its whole length, owing to its taking a sinuous course, the fibres being interwoven one with another : their walls are lignified and pitted. the cell-contents are reduced or absent.

c. **Xylem-parenchyma**, which is to be found more especially around the vessels near the central limit of the bundle. The phenomenon of **thyloses** is the result of the encroachment of these cells on the cavity of the vessels. The normal individual cells are oblong with square ends, they have cellulose walls, and retain their protoplasmic contents.

v. The central conjunctive tissue, **pith**, is composed of parenchymatous cells, with thin walls consisting of cellulose the walls are slightly pitted : these cells have lost their protoplasmic contents in many cases, and especially near the centre of the stem. Occasional **resin-passages** may be found in the pith.

* * * Apical Bud.

1. Take the apical bud of a young plant, or of a young lateral branch of the Sunflower, and cut longitudinal median sections ; treat with potash, and mount in glycerine : a better method is to treat with "eau de javelle," and mount as directed on p. 35 : examine with a low power, and then observe :—

1. That the axis ends in a naked broadly-conical **apex** (*punctum vegetationis*), which is surrounded and enveloped by—

2. **Leaves** : these may be observed in various stages of development, the youngest being nearest to the apex : their order of development, is thus **acropetal**. The surfaces of the older leaves are covered with—

3. **Hairs**, which are absent from the apical cone and the

youngest leaves, the hairs being developed subsequently to the leaves themselves.

Note (with a high power) that the apical cone itself consists of thin-walled cells with plentiful protoplasm, which are smaller than the cells of the mature tissues already studied, and are in a state of active division, *i.e.* they are **merismatic**. Observe further that the newly-formed cell-walls cut the pre-existing cell-walls at right angles, and that the two parts of the cells thus divided are apparently equal to one another. A com-

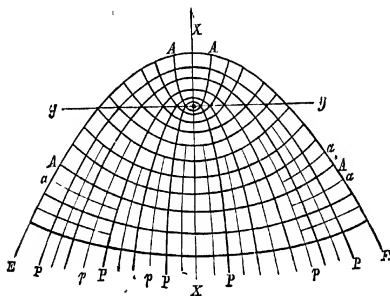


FIG 2—Diagram illustrating the plan of arrangement of cell-walls in the apex of the stem of an Angiosperm. *x, x* = longitudinal axis, which is the organic axis of the stem. *E, E* = external surface *P, P, P, P* are the periclinal curves *A, A* = the anticlinal curves which cut these at right angles *p, p* = incomplete periclinals *a, a* = incomplete anticlinals. The dermatogen is represented by the space between the outer surface, *E, E*, and the outermost periclinal, *P, P*. (After Sachs)

parison of the general arrangement of the cell-walls with the diagram shown in Fig. 2 will help to make clear the arrangement of these cell-walls: in drawing the comparison, however, it must not be forgotten that Fig. 2 is a diagram, and cannot be expected to apply in detail.

The whole merismatic mass is differentiated into parts, which may be distinguished more or less clearly from one another, and it will be easy to trace their continuity with the several tissue-systems of the stem and leaves, of which in fact they

are the **formative tissues**. We may thus distinguish the following :—

1. The **dermatogen**, as a single continuous layer of cells, which divide only in a direction perpendicular to the external surface of the organ (stem or leaf), which it covers completely. It is easily seen to be continuous with the **epidermis**, of which it is the formative layer. Within this is a solid mass of tissue, which looks for the most part dark, owing to its being permeated by intercellular spaces filled with air. It is traversed at a short distance from the external surface by transparent, longitudinal bands of—

2. **Desmogen**, which is the formative tissue of the vascular bundles. Trace its continuity with these. Between the desmogen bands and the dermatogen lies—

3. The formative tissue of the cortex (**periblem**) which is (partially at least) characterized by dark-looking intercellular spaces.

4. Centrally lies a dark bulky cylinder, which is continuous with, and formative of, the **pith**.

Observe carefully the mode of **origin of the leaves**. They appear at the periphery of the cone as protuberances of the dermatogen and of the subjacent cells: the divisions in the dermatogen are all anticlinal, those in the lower layer are both periclinal and anticlinal. (Compare Fig. 3.) As they increase in size their internal tissues become differentiated into (1) desmogen, which is subsequently connected with that of the stem, and (2) tissue with intercellular spaces, which is continuous with the cortex. At the same time single cells of the dermatogen grow out, and divide, so as to form the conical multicellular hairs, which cover the surfaces of the leaves. In the older leaves of the bud the development of the emergences around and below the bases of these hairs may be traced. these are not represented in the diagram (Fig. 3).

Note on passing back from the apex towards the more differentiated part of the stem a gradual increase in length of the cells, corresponding to the **gradual extension** of the internodes, while in the first elongated internode of the stem below the bud this is very marked. Observe also the various stages of

the process of **vacuolization** of the protoplasm ; this will be best seen in sections stained with hæmatoxylin, and mounted in Canada balsam.

Apical buds of the Jerusalem Artichoke (*Helianthus tuberosus*) may be used instead of *H. annuus*, and they have the advantage of being vegetative buds. Whereas the Sunflower flowers

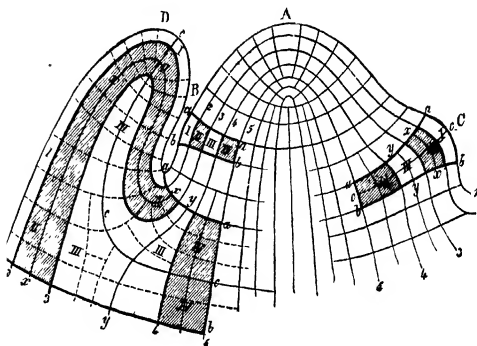


FIG 3.—Diagram illustrating the mode of origin of leaves (with alternate arrangement) from the growing point of a Phacelotogon. A, apex of the growing point ; B, C, D, various stages in the origin of a new leaf. The Arabic numbers, and shading, indicate how the individual layers of cells take part in the origin of the new leaf. It is clearly indicated in this diagram that no periclinial divisions appear in the cells of the dermatogen. (After Sachs.)

early, the Artichoke does not flower at all in this country : thus the complications which attend the formation of flowers will be avoided.

Buds of the Common Lilac (*Syringa vulgaris*) also afford good material : various other buds may be used, allowance being made for difference of detail owing to the various arrangement of the leaves, &c.

STEM OF CASTOR OIL. (*Ricinus communis*).

For comparison with the Sunflower, transverse sections should also be cut from the young stem of *Ricinus communis*: the material should be hardened in alcohol. Mount some of the sections in chlor-zinc-iodine, others in glycerine after staining with acid aniline sulphate. Examine first with a low, then with a high power. Note the following tissues:—

1. The **epidermis**, a regular layer, disturbed occasionally by the occurrence of large rounded cells: here and there are stomata.

2. The **cortex**, differentiated into two bands, an outer denser slightly collenchymatous band, and an inner zone with thinner walls and larger intercellular spaces. The **endodermis** has little to distinguish it but the presence of copious starch.

3. In the **stele** note the continuous ring of **cambial activity**. If the stem be young, the primary bundles will still be isolated. Examine carefully the **cambium** of one of these bundles. Its constituents are cells arranged in radial rows, with thin cellulose walls (blue chlor-zinc-iodine), and plentiful protoplasmic contents. the tangential walls are the thinnest, hence we may conclude that the most recent divisions have been in this direction, and have been repeated. Occasionally traces of recent radial division will be found, but this is less common. The form of the individual cells varies from oblong to square, as seen in transverse section: in the former case the longer axis is tangential. Trace the radial series outwards into the phloem, and inwards into the xylem. Note how, in passing from the cambium to the phloem or xylem, the cells divide, and how the form of the individual cells is modified. Hence we may draw conclusions as to the development of different tissue-elements from the originally uniform cells of the cambium.

Observe how this **cambial activity** extends across the primary medullary rays, as the **inter-fascicular cambium**, which thus completes the ring.

If the stem be more advanced, the result of its activity will be to form tracts of xylem and phloem, which together with the primary bundles form a continuous ring of vascular tissue sur-

rounding the central **pith**. The original bundles may still be identified by their wedges of primary xylem projecting into the pith.

The **pericycle** is partially sclerotic opposite the vascular bundles, but less developed than in the Sunflower.

STEM OF CUCUMBER (*Cucumis sativa*).

1. For comparison with the stem of the Sunflower, and for detailed study of sieve-tubes, sections should be cut from the stem of the Cucumber. Mount some in chlor-zinc-iodine, others in glycerine: others may be stained with eosin and mounted in glycerine.* The material should have been hardened in alcohol. It should be remembered that the Cucumber is a climber, and it shows structural features common in such plants.

Examine first with a low power, and observe.—

1. The **epidermis**, regular, but interrupted by stomata, and by the bases of large hairs.

2. The **cortex** of varying thickness, it is thickest opposite the ridges of the stem: opposite the furrows it narrows down to very few layers. Opposite the ridges it is differentiated into an outer arc of very characteristic collenchyma, and an inner zone of thin-walled parenchyma. Opposite the furrows the latter only is present. The cortex abuts on a strongly-marked zone of sclerenchyma: its innermost layer forming an ill-defined thin-walled starch-sheath—the **endodermis**.

3. The **stele** is defined by a continuous band of **sclerenchyma**, belonging to the broad pericycle, the inner part of which consists of thin-walled parenchyma. The vascular bundles, embedded in the copious conjunctive parenchyma, form two series, of which those of the inner are the larger. The pith is usually fistular.

Examine one of the larger bundles in detail, and note that it is **bicollateral**, *i.e.* that there is a strand of phloem on the inside as well as on the outside of the xylem; the **protoxylem** is **endarch**, *i.e.* at the inner margin of the latter. The xylem is characterized by **unusually large vessels**, which often show

thyloses. The phloem resembles that of *Helianthus*, but has larger constituents. Observe—

i. The **sieve-tubes**, elements with large lumen, which are sometimes traversed by transverse **sieve-plates**; these have a punctate appearance, due to perforation by the **sieve-pores**. The contents of the sieve-tubes stain deeply with eosin.

ii. The **companion-cells**, small cells attached to the sides of the sieve-tubes. They are sister cells of the sieve-tubes, and still appear as though cut off from them by a longitudinal wall.

iii. The **phloem-parenchyma**, which composes the rest of the phloem.

In the sections treated with chlor-zinc-iodine all the walls of the phloem turn blue (cellulose), but the sieve-plates appear yellow or brown, owing to the contents massed around them. In order to see the pores of the sieve-plates, transverse sections may be stained with Bismarck brown, after treatment with "eau-de-Javelle" or potash, to remove the contents.

II. Cut longitudinal sections through the soft bast either radial or tangential sections will do. Mount some in iodine solution. The transverse **sieve-plates** will be brought into prominence by the deep yellowish brown staining of the mass of substance, which surrounds them: this may consist of—

a. A **callus-mass** which surrounds, and often completely invests the sieve-plate: the size of the callus-mass is variable according to season, age, &c., being greatest in autumn, and in old sieve-tubes.

b. The **slimy contents** which are usually collected in close contact with the sieve-plate (or with the callus, if present), and more especially on its upper side. In many cases plasmolysis, acting on the delicate protoplasmic lining of the tubes, will have caused contraction from the lateral walls.

Note, i. the **oblong form** of the segments composing the sieve-tubes.

ii. The **companion-cells**, with granular protoplasm, and nucleus.

iii. **Phloem-parenchyma** of similar form to the segments of the sieve-tubes.

Other sections should be stained with eosin, then washed and

mounted in glycerine. The sieve-tubes will be readily seen, as their contents will be stained deeply. In these sections note especially the stained threads which traverse the pores of the sieve-plates.

III. Treat some fresh longitudinal sections with iodine, then dry off the superfluous fluid with blotting-paper, and mount in a single drop of strong sulphuric acid. The cellulose walls and callus will swell ; the protoplasm will contract. Look carefully over the protoplasmic contents of the sieve-tubes for the points where sieve-plates have been ; here it will be found that fine strings of protoplasm, which passed through the sieve-plate, connect the protoplasmic masses on opposite sides of the sieve with one another. By this reaction the **continuity of protoplasm** through the sieve is demonstrated.

It will be noted that the sieve-tubes of *Cucumis* closely resemble those of *Helianthus*, the sieve-plates being transverse and simple. This is the usual type of sieve-tube to be found in **primary phloem**, and generally in herbaceous stems of Angiosperms. In the **secondary phloem** of ligneous stems a more complicated type of sieve-tube is frequently found. This will be studied in the stem of the Lime.

STEM—AQUATIC TYPE

A. Note the cylindrical smooth stem of *Veronica Beccabunga*, bearing decussate pairs of simple leaves, separated by long internodes.

Cut transverse sections of the internode, preferably from the creeping rhizome. Mount some in glycerine, others in chlor-zinc-iodine, and, examining first with a low power, observe—

1. A well-marked **epidermis**.
2. The **cortex**, a broad zone consisting of—
 - a. A peripheral, slightly collenchymatous band.
 - b. A broad band of parenchyma, with large intercellular spaces, and rounded cells.
 - c. A well-defined **endodermis**, with cutinised radial walls.
3. The **stele**, consisting of—
 - a. A central **pith**, which also has large lacunæ.
 - b. A **continuous ring of xylem**, not separated into strands belonging to distinct vascular bundles. Note the crushed protoxylem at its inner margin (endarch).
 - c. The **phloem**, which is also a continuous ring, with small sieve-tubes in groups, among the large-celled phloem-parenchyma.
 - d. Between the xylem and phloem lies a **cambium**, which is only slightly active.

The continuous vascular ring, and the lacunar parenchyma are characteristic of aquatic plants : but this stem is not a highly specialized type.

B. Note the cylindrical smooth stem of the Mares-tail (*Hippuris vulgaris*), bearing whorls of simple leaves. Cut the stem transversely, and note the central vascular cylinder, which is easily seen with the naked eye, and the numerous large lacunar spaces present in the cortex.

For the microscopic work fresh material may be used. If the material has been kept in alcohol, which gives better results, the sections should, after being cut, be allowed to swell in water before mounting.

I. Cut transverse sections of an internode of the stem of *Hippuris vulgaris*: mount in glycerine and examine with a low power. Observe:—

1. A well-marked **epidermis** with cuticle and occasional stomata. Here and there are to be seen radiating scale-hairs. These occur especially in the axils of the leaves.

2. The **cortex**, consisting of—

a. **Parenchyma**, a broad band of thin-walled, chlorophyll-containing cells, with large intercellular spaces, or **lacunae**, which are much smaller towards its inner limit. Here the cells are slightly collenchymatous.

b. The **endodermis**, a well-marked single layer of barrel-shaped cells; note the closely-fitting radial walls, which bear the **cutinized dot**, typical of endodermis cells.

3. The **stele**, or central cylinder, composed of a more or less copious central pith, surrounded by a ring of vascular tissue. The vascular ring is again in this case continuous, and not broken up into separate vascular bundles. Examine in detail:—

a. The **xylem**, forming the inner portion of the vascular ring, and consisting of annular and spiral vessels, intermixed with parenchyma. The protoxylem elements may be found more or less disorganized at its inner margin.

b. The **phloem**, forming the outer portion of the vascular ring; the sieve-tubes and companion cells are difficult to distinguish: the latter are marked by their small size and dense contents.

c. The **pericycle**, which is badly defined from the phloem.

Hippuris thus shows more plainly than *Veronica* the reduced vascular construction commonly found in water-growing plants. The lacunae also are of larger size.

II. Take a terminal bud of *Hippuris*: remove from it the largest external leaves, and then dissect off the inner and smaller leaves with needles in a drop of water on a glass slide: in the centre of the bud will be found the elongated conical and

colourless **apical cone**. Examine it under a low power the smooth cylindrical apical cone will be well seen, the inner tissues of it being marked by a reticulum of dark lines. these are the **intercellular spaces** filled with air.

Note especially the **leaves**, which appear as rounded outgrowths laterally on the axis the larger ones are seated lower down the axis, and successively smaller ones are seen as the apex is approached.

III. Cut median longitudinal sections of the apical bud of *Hippuris*, so as to pass through the elongated **apical cone**; treat with potash, or with "eau de javelle." and mount in dilute glycerine. Examine first with a low power, and observe—

1. The **axis**, which is wide below, but tapers upwards to the rather elongated **apical cone** (*punctum vegetationis*). The axis is composed of the several tissues already noticed. Note especially in the lower part of the section—

a The rectangular **intercellular spaces**, divided transversely by **diaphragms** at the nodes.

b. The axile **vascular cylinder**, or **stele**, which may be followed far up into the apical cone, and from it vascular strands depart to the leaves.

2. The **leaves**, diminishing in size towards the apex. Note the **scale-hairs** about the bases of the leaves.

Put on a high power, and examine the apical cone. Note—

i. The **dermatogen**, a continuous layer of cells, which covers the apical cone externally. Trace it backwards from the apex it will be seen to give rise to the **epidermis**.

ii. The **periblem**, consisting of 4-5 layers of cells, which may be traced backwards, and be thus shown to give rise to the bulky **cortex**.

iii. A central cylinder of **plerome**, which is continuous with, and gives rise to, the **vascular cylinder** or **stele**. (Compare the diagram Fig. 2, p. 57.)

Note that the **leaves** originate from the outgrowth of the dermatogen and periblem, the plerome taking no part in their formation.

STEM—ARBOREOUS TYPE

1. Note the following external characters of a lateral twig of the Lime (*Tilia Europaea*), of the current year, taken in May, when about three inches long. The **axis** is cylindrical, smooth, and pale coloured—it is continuous with the brown **axis** of the preceding year, the junction being marked by the scars of the protective **bud-scales** some of which may still persist.

The **leaves** are alternate, and petiolate; they are disposed so as to give a dorsi-ventral character to the shoot. At the base of each petiole note right and left, two **stipules**, or the **scars** of their insertion: they are protective in the bud, and fall off soon after the buds burst in spring. Buds are found in the axils of the leaves.

Examine older shoots, and note the successive increments of growth of former years, limited by similar scars of bud-scales. The brown colour is due to formation of cork, while the surface is marked by numerous small brown excrescences, the **lenticels**.

11. Cut thin transverse sections of the youngest elongated internode of a young twig of the current year. mount some in glycerine, others in chlor-zinc-iodine, and examine with a low power. Observe the following tissues in succession starting from the outside:—

1. The **epidermis**, a single layer of cells.
2. The **cortex**, a broad parenchymatous band, limited internally by a well-marked **starch-sheath** or **endodermis** (blue with chlor-zinc-iodine).
3. The **stele**, occupying the centre, and consisting of a **vascular ring** surrounding a massive central **pith**. The vascular ring is of unequal thickness, the broader regions indicate the ill-defined **vascular bundles**.

Examine the sections with a high power, and observe as follows —

- i. The **epidermis** consists of uniform cells with convex cutinised outer walls.
- ii. The **cortex** is composed of a slightly **collenchymatous** outer zone of small cells, and an inner thin-walled parenchyma note the large cells with **mucilaginous walls**, which have swollen so as to fill the cell-cavity. The inner limit of the cortex is the **endodermis** a regular layer of starch-containing cells.
- iii. In the **stele** observe, next to the endodermis, one or two irregular layers of the **pericycle**, large parenchymatous cells, without starch. The vascular ring is composed of a peripheral band of immature **phloem**, and an inner band of immature **xylem**, between which may already be seen the zone of **cambium**: the result of its activity will be **secondary phloem** and **xylem**, the arrangement and structure of which will be studied in sections of the older stem. The central **pith** presents characters similar to those of the cortex.

In arboreous Dicotyledons the activity of the cambium begins so near to the primary meristem of the apex, that it is difficult to distinguish with certainty between the primary vascular tissues, derived from the apical meristem, and the secondary which arise from the cambium. As a consequence of this secondary growth the distinctions between the different parts of the primary tissues are obscured.

III. Cut a four-year old twig of Lime transversely. The age of a twig may be judged externally by counting backwards the annual increments of growth from the apex.

Smooth the cut surface with a razor: examine with a lens, and observe.—

1. The **pith**, which occupies the organic centre of the stem. Its position does not, as a rule, coincide with the geometrical centre. Externally to this lies—

2. The **xylem**, which is here a broad yellowish band, clearly marked off into a succession of concentric rings; these, as a rule, correspond in number to the years of the twig (**annual rings**).

3. The **cambium** lies at the outer limit of the xylem, but it

will hardly be recognized as a definite band of tissue under a simple lens, since it is a very narrow zone its position may frequently be recognized by the rupture of the tissues, the walls of the cambium being thin and easily broken. Outside this is—

4 The **phloem**, which is a much narrower band than the xylem, and is also marked off, though less distinctly, into concentric rings. The phloem appears to consist of darker coloured wedges, separated laterally from one another by lighter coloured masses of tissues, which are the **medullary rays**. Trace these rays inwards into the xylem, where they diminish greatly in width some of them may be continued through to the pith—the principal medullary rays. Outside the phloem lie—

5. The **cortical tissue** and **cork**, which are usually of insignificant bulk, compared with that of the vascular tissues.

The general plan of the process of secondary thickening, and the relation of the secondary tissues to the primary arrangement are made clear by means of the diagrams *A, B, C*, of Fig. 4.

IV From a twig of the third or fourth year, cut transverse sections the sections need not be complete ones, but should be such as to include all the tissues from the periphery to the centre. Mount some in glycerine, others in chlor-zinc-iodine, and examine first with a low power. Starting from the periphery, observe —

1. A brown band of tabular cells, arranged in radial rows this is the **cork**: for its origin see below under the heading of Cork (p. 79).

2 The **cortical parenchyma**: the collenchymatous outer region persists, its cells showing lateral extension, and division by radial walls, so as to keep pace with the increased bulk within. The inner cortex is crushed by the pressure from within many of its cells contain large crystals of calcium oxalate. The endodermis is no longer recognizable. Compare this with the section of the young stem.

3. The **phloem**, separated into broad wedges by the widened medullary rays, as above noted. Examine one wedge carefully, and observe that it consists of irregular alternate zones of thin-

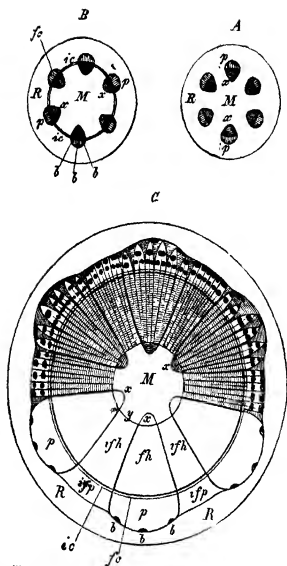


FIG. 4.—Diagrams illustrating secondary growth in thickness in a typical Dicotyledon. the diagrams are based on drawings of transverse sections of the hypocotyl of *Ricinus*. A, B, C, represent the condition of the stem at different stages of development: A, before the origin of the interfascicular cambium, B, after the interfascicular cambium has been formed, C, after the cambium has been active for some time, producing internally a broad ring of secondary xylem, externally a narrow ring of secondary phloem. R=primary cortex; M=pith; p=phloem; x=primary xylem; b, b, b=three groups of bast fibres at the periphery of the phloem; ic=interfascicular cambium; ic=interfascicular cambium; fh=wood developed from the fascicular cambium; fh=wood developed from the interfascicular cambium; fh=secondary bast developed from the interfascicular cambium. By the intercalation of the secondary xylem and secondary phloem, the primary groups of bast fibres, b, b, b, are removed a considerable distance from one another. Note also in C the principal medullary rays, which extend the whole distance from the periphery of the ring to the pith, while the secondary medullary rays only extend through part of that distance. (After Sachs.)

walled tissues, and thick-walled elements the latter are the **phloem-fibres**. At its inner limit observe—

4 The **cambium**, a band of narrower cells in regular radial series, which may be traced outwards into the phloem, and inwards into the xylem.

5. The **xylem**, or wood, a broad zone occupying the greater part of the section. It is composed of thick-walled tissues and is differentiated into successive **annual rings**, distinguished by the denser autumn-formed wood and less dense spring-wood.

6. The **pith**, as before.

7 The **medullary rays**. select one **principal ray**, and follow it out from the pith to the cortex, noting especially how it widens out in the region of the phloem into a broad tract. Note smaller rays between these, which, starting from the cambium, end blindly outwards in the phloem, and inwards in the xylem these are the secondary rays.

Put on a high power, and examine the following tissues in detail —

a In the **phloem** the **phloem-fibres** will be seen in irregular zones of cells, polygonal in section, with walls so thickened as almost to obliterate the lumen: they are lignified, and show the middle lamella well differentiated from the layers of thickening. The thinner-walled zones between these consist of large elements, usually with sparing contents—the **sieve-tubes** sieve-plates are occasionally seen on the oblique terminal walls. The small **companion cells**, with dense contents are always in contact with the sieve-tubes. **Phloem-parenchyma** cells of intermediate size, with protoplasmic contents, form a single more or less continuous layer bordering the fibres on either side. these cells sometimes contain crystals.

b The **cambium**, which appears here as a narrow band of cells at the inner limit of the phloem, with thin walls and abundant protoplasmic contents. Careful observation of a good section will show their tabular form, their arrangement in radial rows, and that their radial walls are thicker than the tangential. Such structure indicates repeated division by tangential walls.

c. In the **xylem** (excluding the medullary rays) the most

prominent objects are the **tracheæ**, recognized by their large lumen ; they occur isolated, or in groups, and are limited by thickened and pitted walls, and are without contents ; the smaller elements without contents and with smoother walls may be **wood-fibres**. The **xylem-parenchyma**, consisting of thick-walled cells with living contents, is distributed throughout the wood. Examine the innermost region of the wood note the radial rows of vessels, and how the innermost of them may be crushed. these are the **protoxylem** elements of the primary wood.

Observe the alternating denser and less dense zones of the wood. In the denser wood, which is formed in **autumn**, the larger vessels are absent and the elements are on the average smaller. In the less dense **spring wood**, immediately outside the autumn wood, large vessels are present : and thus the annual rings are defined.

d. The **medullary rays**, in their course through the xylem, consist of radially elongated, lignified, thick walled, and pitted elements, with living contents. They have special cambium-cells of their own. passing outwards to the phloem, the cells assume thin, cellulose walls, and show active tangential growth and division : thus producing the broad inverted wedges above noted.

e. The **pith**, consisting mainly of thin-walled cells, with cellulose walls, and intercellular spaces. Here and there are smaller rounded cells, with thicker walls, and more plentiful contents. Mucilage cells are present.

V. Cut radial sections from a four-year-old stem of Lime, and mount in glycerine. Other sections may be mounted in chlor-zinc-iodine, and these will perhaps be found the most useful ; examine first under a low power.

It will be found difficult to cut good sections so as to include the whole radial surface. it is therefore better not to attempt it, but to study the several structures in a number of successive sections, each extending over only a part of the radial surface.

Starting from the outside, observe the same succession of tissues as already seen in the transverse sections, but the

epidermis has usually been thrown off, owing to the formation of—

1 The **band of cork**, which presents the same appearance as in the transverse section. (See p. 79, &c.)

2. Within this is the **cortical tissue**, with large mucilage cells, and crystals.

3 The **phloem**, consisting of alternating bands of thin-walled tissue and phloem-fibres.

4 The **cambium**, an ill-defined streak of tissue, at the inner limit of the phloem.

5. The **xylem**, with thick lignified walls, the **vessels** appearing as large tubular cavities.

6 If the section be truly radial the **medullary rays** will appear as narrow bands of tissue, following the plane of the section.

7 The **pith**, consisting of parenchymatous cells as before

Examine the following tissues in detail with a high power.

a. In the **phloem**, the **phloem fibres** are to be observed as narrow, elongated, finely pointed cells, with very thick walls, forming compact strands. In the spaces between these observe the **sieve-tubes**—broad tubes with thin cellulose walls, within which the contents appear massed at intervals, thus indicating the positions of the oblique terminal walls, which bear the **sieve-plates**: they are here presented in surface view. Try to observe the sieve-plates—dotted areas, three to five of which are present on each oblique wall—they are best seen just outside the cambium. Closely adjoining the sieve-tubes are the narrow **companion cells**, and the shorter cells of the **phloem-parenchyma**, which are difficult to distinguish from one another in radial section.

Stain some sections with eosin for a few minutes, carefully washing out the superfluous stain with water—this will bring the sieve-tubes into prominence, owing to the deep staining of their contents.

b. The **cambium**, which appears here as a narrow band of cells with thin walls, and abundant protoplasmic contents. The form of the cambial cells is difficult to make out, but a careful observation of a good section will lead to the conclusion that

the form of the cell as seen in the radial section is oblong and very narrow, with square ends. Compare the diagrammatic figure (p 77).

c. In the **xylem**, excluding for the present the medullary rays, observe the following elements, all of which have lignified walls, viz.,—

Vessels of various orders, which may be grouped as **spiral vessels (protoxylem)** found at the central part of the xylem, next the pith they are usually more or less disorganized.

Most of the tracheæ of the secondary wood have both pitted and spiral markings on the same wall, but a few **pitted vessels** without the spiral are found next the protoxylem.

Note in the larger **tracheæ** the points where the transverse or oblique septa have been partially or completely absorbed. Those tracheæ in which this occurs are designated **vessels**; in others no perforation takes place, these are called **tracheides**. The **xylem-fibres** are distributed generally throughout the wood: they are seen to be elongated, pointed elements with small simple pits on their walls. The **xylem-parenchyma** consists of longitudinal strands of short cells; mostly with transverse ends the walls are lignified and pitted, and the cells have living contents, often also with starch.

d. Examine the **medullary rays** in the xylem they are composed of oblong cells, with their longer axes horizontal, arranged like bricks in a wall in characters they resemble xylem-parenchyma.

In the phloem, the cells of the ray are polygonal with thin cellulose walls. Many mucilage cells are also present.

VI. Cut tangential sections through the xylem of a stem of Lime, an inch or so in diameter. treat with acid solution of aniline sulphate, and mount in glycerine others should be mounted in chlor-zinc-iodine. Observe first with the low power —

1. The **medullary rays** of varying size they are tracts composed of small rounded cells, and are continuous only for a short distance up and down the stem. Some will consist of a single layer only, others may be two, three, or more layers thick. Examine one of the latter with a high power, and note

the roundish cells with thickened lignified and stratified walls. they contain protoplasm, and often in the autumn starch. Observe the small triangular intercellular spaces between them.

By comparing transverse, radial, and tangential sections it will be seen that the medullary rays are narrow parenchymatous plates, radiating outwards from the centre, and that they do not extend far up or down the stem.

2. The **xylem-parenchyma** as before described note that the cells are arranged in vertically disposed, spindle-shaped groups.

3. The **wood-fibres**, elongated, pointed, spindle-shaped cells, with smooth walls, occasionally marked by simple pits.

4 The **tracheæ**, as before note that they also are derived from spindle-shaped cells, and observe specially the perforations of the septa in the vessels, which are better seen here than in the radial section. In the tracheides the septa are not perforated.

VII. Cut tangential sections of the phloem, so as to traverse the youngest tissues just outside the cambium stain with eosin, wash thoroughly in water, and mount in glycerine. observe first with a low power —

1. The **medullary rays**, which on the average are here much broader than in the xylem. their cell-walls are thin and unlignified.

2 The **phloem-fibres**, which are very long and pointed, and disposed in irregular sinuous strands; their walls are thick and lignified.

With the high power examine the softer tissues, and observe—

3 The **sieve-tubes** marked by the pink staining of their contents by the eosin. They are wide tubes, derived from longitudinal rows of spindle-shaped cells. examine especially the oblique terminal walls; these bear the **sieve-plates** already observed in surface view in the radial section here they are seen on edge.

Focus carefully upon the terminal wall, and note the thickened portions which separate the thinner sieve-plates from one another; the sieve-plates themselves appear minutely dotted, owing to their perforations. Note that the contents of the sieve-tubes are massed in the neighbourhood of the septa.

4. The **companion cells**, very narrow cells lying side by side with the sieve-tubes, and of the same length as their segments.

5. **Phloem-parenchyma**, short cells with transverse or oblique terminal walls, arranged in spindle-shaped groups: many of the cells contain crystals of calcium oxalate.

VIII. Cut tangential sections through the **cambium** of the stem of Lime; treat with dilute potash, or "eau de javelle," and mount in glycerine. Examine first with a low power, and note that the general arrangement is similar to that already seen in tangential sections through the mature tissues, also that the form of the cells, in each part of the cambium-zone, is like the average form of the elements of the mature portion of wood or bast, which borders on it in a radial direction. Thus the cambium is differentiated into—

1. Cambium of medullary rays, which appears to consist of roundish cells, resembling cells of the mature medullary rays in form.

2. Cambium, from which all the other tissues are derived, the cells of which have a prismatic form.

To gain a clear idea of the process of secondary thickening the actual form of the cambium-cells and their arrangement must be recognized: as stated above (p. 6), it is necessary, in order to fully realise the form of a cell as a solid body, to cut sections in three directions at right angles to one another: the cambium-cells have now been seen in transverse, radial, and tangential sections, and the results are represented diagrammatically in Fig 5, A, B, C, which are based upon results of Sanio's investigations of *Pinus*, but the main points are the same for Dicotyledons.

Fig. 5, A, shows diagrammatically four radial rows of cambium-cells (1, 2, 3, 4), as seen in transverse section: of these row (2) is a medullary ray. Note in row (3) the single initial cell (*i*), oblong in transverse section, and the shorter diameter placed radially; accordingly to Sanio's law of cambial division there is only one such initial in each radial row: from this successive segments (*ww*) which go to form wood have been cut off on the inner side, others (*b, b, b*) which go to form bast on the outer side: each is represented in the diagram as

dividing into two by a periclinal wall; this is typically the case in *Pinus*, but the division is not so regular in Dicotyledons. In row (1) is represented a segment (w'), recently cut off from the initial cell on the side next the wood, in which this division has

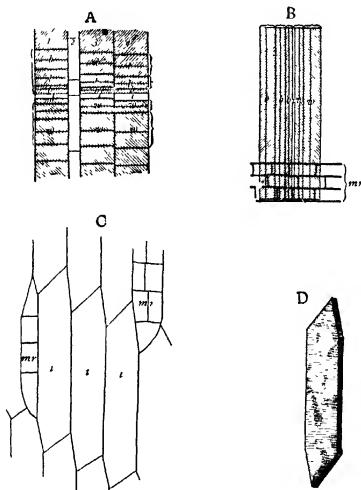


FIG 5

not yet taken place; in row (4) there is a similar undivided segment (b') which, after division will go to form bast.

Fig. 5, B, shows diagrammatically the arrangement of the cells of one of these rows (3), as seen in radial section. the length of the cells is much greater than their width, and the ends are square: i is, as before, the initial cell of the row: ww , pairs of cells formative of wood: b, b, b , pairs of cells formative

of bast : *mr* is a medullary ray put in so as to show the relative position and form of the cells.

Fig. 5, C, represents the appearance of the cambium cells *i*, *i*, *i*, in tangential section. they are obliquely pointed, and their width corresponds to that shown in A. *mr*, as before, the medullary rays.

Fig. 5, D, shows the form of a single isolated cambium-cell as a solid body, drawn to the same scale as the other figures : if such a cell be cut transversely, radially, or tangentially, it would give the appearance presented by the initial cells (*i*) in Figures A, B, and C.

Taking cells of this form as a starting point, the several tissues above described are derived from them in the following way :—

(i.) **Phloem**—*a. Sieve-tubes*, by lateral distension, and conversion of the oblique walls into sieve-plates.

b. Parenchyma, by division of the cells by transverse septa.

c. Fibres, by elongation and interweaving of cells, the width of the cells at the same time being relatively reduced : the end of the cells slide past one another as the cells elongate.

(ii) **Xylem**—*a. Vessels*, by lateral distension, and absorption of cell-contents, and of the oblique walls.

b. Parenchyma, by division of the cells by transverse septa.

c. Fibres, by elongation and interweaving of the cells, while the width of the individual cells is relatively reduced.

Observe intermediate stages between cambium-cells and these several mature-tissues : this may best be done in sections cut from stems in early summer.

IX. Treat some small pieces of the wood of the Lime with a small quantity of Schulze's macerating fluid (see Appendix A) in a test tube, and warm gently till the tissues break up, and

the several constituents begin to separate: then wash with water, and mount a very small quantity in water or glycerine.

Some at least of the constituents will be found lying separately, or may be detached by slight pressure on the cover-slip: the true form of the **wood-fibres**, as elongated, spindle-shaped cells, will now be seen. Note also **tracheæ** of the various types above described, the **xylem-parenchyma**, and the **parenchyma** of medullary rays.

Pieces of some harder wood, such as the Elm or Oak, may be similarly treated. Note in these that the wood-fibres are more numerous and longer, and in the Oak the great width of the vessels. The wood-parenchyma is also well seen in these woods.

X. Examine with the naked eye or with a lens complete transverse sections of old stems of various trees, *e.g.* Lime, Elm, Laburnum, and Oak. Note that in the Lime there is no obvious differentiation of the wood. In the Elm, Laburnum and Oak, the central portion of the wood is darker in colour and harder. this is the **duramen** or **heartwood**. The peripheral part is paler in colour and softer. this is the **alburnum** or **sapwood**. Compare also the black duramen and pale alburnum of Ebony, the red duramen of Logwood, &c., &c.

CORK, BARK, AND LENTICELS

XI. The presence of cork and lenticels has been already noted above. to study the origin of the cork in the Lime, examine transverse sections taken from twigs of the current year, at a point where the colour of the stem changes from green to pale brown. Note the superficial **epidermis**, as above described: here and there a **lenticel** may be traversed, appearing as a superficial prominence. these are better observed in the Elder (see below, p. 81). The **cork-formation**, which is called collectively the **periderm** is a secondary formation: it makes its appearance in the cortex immediately below the epidermis, where the cells undergo repeated divisions parallel to the surface: as a result its cells are arranged in radial rows, without intercellular spaces. Select a thin part of the section for

special study of these radial rows, and note in each the following succession of tissues, passing from without inwards.—

1. A series of **cork-cells**, which are rectangular, and, if numerous, are arranged in radial rows. their walls are thin and cutinised; they stain yellowish brown with chlor-zinc-iodine. Protoplasmic contents are absent from the older cells.

2. At least one cell with very small radial diameter, and with protoplasmic contents, and thin cellulose walls. this is the **cork-cambium**, or **phellogen**.

3. Cells with thick cellulose walls, and protoplasmic contents with chlorophyll. no intercellular spaces. this is the **phello-derm**, which is also derived from the cork-cambium.

Treat a thin section with concentrated sulphuric acid. the walls of all the tissues will swell, and gradually lose their sharpness of outline, with the exception of the cuticularized outer wall of the **epidermis**, and the **cork**, both of which resist the action of the acid. A similar result may be obtained on treatment with strong chromic acid.

By comparing sections of twigs of various ages, starting from the youngest, the following facts may be established—

i. The cork-cambium appears in the layer of cortical cells immediately below the epidermis.

ii. These cells divide parallel to the external surface of the stem.

iii. The result of successive divisions in this direction is the formation of secondary tissues, which develop externally as cork, internally as phelloderm.

iv. The true cork-cambium consists of only a single cell in each radial row, from which, by successive division, all these secondary tissues, are derived: compare cambium of vascular bundles. (See above, p. 76, &c.)

v. The cells of the cork-cambium occasionally divide radially.

The diagram (Fig. 6) will help to make this plain.

In older stems of the Lime, the cork will be found as a brown superficial band, but the epidermis will have dried and peeled off.

Successive bands of similar cork may be formed in the inner

cortex, pericycle, or even traversing the phloem, cutting off successive bands of tissue outside them. These bands, together with the cork, are called **bark**. As examples, examine old stems of Elder, Vine, &c.

XII. Note on a stem of Elder of the current year, numerous brown excrescences, scattered over its surface—these are the **lenticels**. Cut transverse sections, so as to pass through one or more of these, and mount in chlor-zinc-iodine. The lenticel is seen to project beyond the general surface of the stem, while

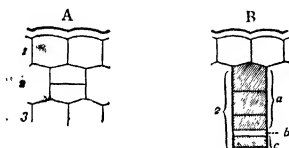


FIG 6—A, B, Diagrams illustrating the formation of periderm in the layer of cells (2) directly below the epidermis (1). A shows the first periclinal division of the hypodermal layer (2). B shows as the result of repeated periclinal divisions a radial row of cells, of which the outer portion (a) is of cork, the innermost portion (c) is the phelloderm—these are separated by a single cell (b), which represents the cork-cambium.

the epidermis curves outward, and if the lenticel be old, it will be ruptured. If the lenticel be young, the epidermis may still be continuous, and a stoma may be found at its centre.

Below the epidermis will be a spongy mass of rounded cells, with large intercellular spaces, which arise from an active cambium below. As the stem grows older, this cambium may be traced as continuous, right and left, with the ordinary cork-cambium. In the autumn the lenticel is closed at its base by an air-tight band of cork. This may again rupture in a succeeding season.

Note on Crystals.

I To investigate the nature of the **crystals** several times observed in the parenchyma of the stem of the Lime, cut

tangential sections of the **phloem** or of the **cortical tissue**, mount in water, and having found one or more crystals—

i. Run some iodine solution under the cover-slip: the crystal is not stained.

ii. Acetic acid . it is not attacked

iii. Dilute nitric acid it is more or ~~less~~ completely dissolved.

iv. Irrigate a fresh preparation with a small quantity of dilute sulphuric acid the crystals will be dissolved, and crystals of a different form (calcium sulphate, which is not readily soluble) may be seen to be formed in the fluid

These reactions, coupled with what can be ascertained from analysis of the ash of the plant, point to the conclusion that these crystals consist of **calcium oxalate**.

II Cut transverse sections of the petiole of some species of *Begonia* ; mount in water, and examine under a low power Here and there will be found large bodies of a ~~more~~ or less distinctly crystalline form occupying the cavities of certain cells Their form is very complicated, and their size variable.

The reagents above applied are to be used : the results will be similar. Thus they also may be shown to consist of calcium oxalate. Crystals giving the above reactions will be found in the tissues of most plants.

· LATICIFEROUS TISSUES

Of all the occasional tissues, which occur in plants the most important are the laticiferous tissues, which are commonly found in certain natural orders, such as the Papaveraceæ, Euphorbiaceæ, Compositæ, &c.

The material for the study of these tissues should be prepared by treatment with alcohol to coagulate the **latex**. Care should be taken to place the material in alcohol **directly it is cut**, or at least the cut surfaces should be wetted with alcohol so as to check the flow of latex from them. If the latex be allowed to escape, the laticiferous tissues are emptied, and are then much less easily traced than when they are full. The best method is perhaps to preserve the **whole plant** without injury in alcohol, in which case the latex will not be lost at all.

Draw from a piece of the fresh stem of *Euphorbia* a drop of latex upon a slide. examine it quickly under the microscope, and observe that the fluid is at first almost uniformly milky, but that in a short time a **coagulum** separates in irregular masses from the more transparent fluid. The coagulation is effected more completely and rapidly on addition of a drop of alcohol.

1. *Laticiferous Vessels.*

1 Cut tangential sections from the phloem of the root of the Dandelion (*Taraxacum officinale*), treat with potash and mount in glycerine, and warm; examine under a low power.

The main constituents of the tissues are parenchymatous cells, with thin walls (**phloem-parenchyma**) **sieve-tubes** are to be met with here and there. The whole mass of tissue is permeated by a ramifying and profusely anastomosing network of **laticiferous vessels**. The communication of these tubes with one another is demonstrated by the continuity of

their coagulated contents (**latex**), which appear brown and granular

The course of the vessels is mainly longitudinal, while lateral, horizontal branches frequently connect the parallel tubes.

With a high power make out more accurately the course of a group of the vessels, and observe especially any indications of breaking down of septa originally present. The development which can be studied in young material, shows that these tubes are the result of cell-fusion.

II. Cut transverse sections of the same ; mount in glycerine, and examine with a low power.

The laticiferous vessels appear circular in transverse section, and have brown contents . they are distributed in groups, which form more or less regular concentric rings round the central xylem. They may be recognized still more distinctly in sections stained with alkannin, or with potassium bichromate.

Note in these sections the presence of **sphere-crystals of inulin** : in the former section they will have been partially or completely dissolved by the treatment with potash. Observe that they are formed quite irrespective of the cell-walls, which are often included in them.

ii. *Laticiferous Cells.*

I. Cut tangential sections of the cortex of *Euphorbia splendens* (other species will do) just outside the vascular ring, and mount in water, or dilute glycerine : or stain with alkannin, and mount in glycerine.

Examine with a low power.

Running through the cortical parenchyma will be seen long tubes, with thick cellulose walls and granular contents. These are the **laticiferous cells**, which differ from the preceding in being developed, not by fusion of originally distinct cells, but by continued apical growth of single cells.

Note cases of **branching** of these cells.

Included in the granular contents are **starch-grains** of peculiar dumb-bell form.

Treat sections with iodine solution, and observe the effect on these bodies.

II. Cut tranverse sections of the same stem, and note the distribution of the laticiferous cells, they may be recognized by their walls, which are thicker than those of the surrounding tissues, and appear circular in section.

III. Separate the whole cortex from a piece of the stem; boil it in potash for about five minutes, and tease out the long laticiferous cells with needles; mount and observe with a low power. They appear as long cylindrical tubes, with thick walls. Observe occasional branching. They are usually broken at the ends, the length of the tubes being greater than that of the parts teased out.

Note.—The parenchymatous tissues of *Euphorbia splendens* contain within the cells numerous **crystals of reserve proteid material**: their form is variable. They stain yellowish brown with iodine solution, and are not readily soluble in salt solution.

LEAF

A.—PETIOLE

Observe that the mature leaf of the Sunflower consists of an upper, flat, expanded portion—the **lamina**, and a lower, narrow stalk—the **petiole**, by which it is inserted on the stem. Note the channelled upper surface of the petiole, and the broad insertion on the stem in the angle between the petiole and the stem may usually be observed an **axillary bud**, or **shoot**.

1. Cut transverse sections of the petiole, and mount in glycerine. The details of structure resemble in many respects those of the young stem, from which the petiole differs in the following points :—

1. The general outline of the section is **semilunar**, the concave being the superior (**adaxial**), while the convex is the inferior surface (**abaxial**). thus the petiole is **dorsi-ventral** whilst the stem is **radially symmetrical**. This property extends also to the arrangement of the vascular bundles, of which the xylem is as a rule directed towards the upper surface of the petiole.

2. The presence of occasional **stomata**; beneath each stoma the collenchyma is replaced by chlorophyll—containing parenchyma with intercellular spaces. Note beneath each stoma an enlarged intercellular space—the air-chamber.

3. The vascular bundles are arranged in a curve following the outline of convex surface of the petiole : there are usually three larger bundles of which one is median, and a varying number of smaller ones.

4. The absence of interfascicular cambium: the bundles

are for a short time at least, open bundles, having an active cambium.

- 5 No general endodermis is present

B — LAMINA

1 Take a piece of the lamina of the leaf of the Sunflower, including the apex — it is important that it should be previously bleached by treatment with alcohol — warm it gently in a mixture of dilute glycerine and potash, and mount in glycerine — examine with a low power, and observe —

1. The **midrib**, with its strongly marked **vascular bundle**, running up to the apex of the leaf, where it terminates abruptly in a mass of glandular tissue.

- 2 Lateral **branch-bundles**—the **ribs** or **nerves**—passing off from it, and forming a network by frequent anastomoses, while some of them run up into and terminate in the serrate projections of the margin of the lamina.

- 3 Smaller branch-bundles sometimes showing **blind endings** in the parenchyma which fills the meshes of the network.

- 4 Examining the specimen more closely, numerous multicellular **hairs** will be seen projecting from the surface, while included in the superficial epidermis, which consists of tabular cells, will be **seen** numerous **stomata** each of these has two **guard-cells**, and their orientation is irregular.

II. As the lamina of the Sunflower is not a convenient one for cutting in section, the Privet (*Ligustrum vulgare*) will be taken.

Good sections may be obtained even from fresh material by holding a piece of lamina between slices of pith ; or by folding the whole lamina repeatedly, and cutting sections from the whole mass. Although the material is fresh, it will be found convenient to keep the razor moist with alcohol while cutting the sections. The direction of section should be such as to cut the midrib transversely : select the thinnest section : mount in glycerine, other sections in chlor-zinc-iodine, and examine first with a low power :—

Note the outline of the section : it is of uniform width, except

where it expands at the midrib, which projects on the lower, or abaxial surface. The midrib is traversed by one large vascular bundle, of semi-circular outline. Note its xylem directed towards the adaxial surface. This is the common disposition of vascular tissues in the lamina.

Starting from the upper surface, examine in succession the tissues composing the thinner parts of the lamina, which will be recognized as follows. —

1. The **upper epidermis**, a single continuous layer of cells with cellulose walls surrounding the protoplasmic body. The superficial wall is slightly thickened, and cutinised. In sections treated with chlor-zinc-iodine the cuticle appears as a continuous yellow film.

2. The **palisade parenchyma**, one or two layers of columnar cells, with their longer axis perpendicular to the outer surface. Each cell is thin-walled, and contains a protoplasmic body, and nucleus, with numerous **chlorophyll-grains**. Note the narrow intercellular spaces intervening between the cells.

3. The **spongy parenchyma**, composed of cells similar to the above, but loosely packed, of very irregular form, and with large intercellular spaces. These two tissues constitute the **mesophyll**. Occasional cells of this tissue contain **crystals** of calcium oxalate.

4. The **lower epidermis**, similar to the upper, but less regular, covers the lower surface. Occasional **glandular hairs** will be found, with their short stalks sunk in depressions of the surface.

5. Numerous smaller **vascular bundles** are found embedded in the mesophyll. Many will be cut obliquely, and their structure will be obscure: those cut transversely will show the xylem towards the upper surface, and phloem towards the lower.

Examine sections of the **stomata** in detail, under a high power. As in the Sunflower, the orientation of these is irregular, and many therefore will be cut obliquely. Having found one cut transversely, note the two small guard-cells, seated towards the outer surface: the pore between them opens into the intercellular spaces of the ventilating system.

III. Cut tangential sections from the upper and lower surfaces

of the leaf of Privet, so as to show the superficial tissues in surface view mount in glycerine, with the outer surface uppermost, and examine the thinnest portion with a high power. Note:—

1. That the **upper epidermis** consists of tabular cells, with thickened and pitted lateral walls, and without any intercellular spaces. each cell has a protoplasmic body and well-marked nucleus. Stomata are absent.

2. That the **lower epidermis** consists of similar tabular cells, with thinner walls. numerous **stomata** are present, distributed without any regularity of orientation. Examine a stoma in detail, in a thin part of the section, and observe the two sausage-shaped **guard-cells**, of equal size. These surround and control the **pore** of the stoma, which will usually be found to be closed in the dead state. Note their protoplasm and nucleus, and numerous **chloroplasts**. Run in an iodine solution, and note the blue colouration, indicating the presence of **starch**, which is absent from other epidermal cells. The **glandular hairs** will be seen, scattered here and there, presenting a rosette-like appearance.

IV. As a good alternative to the Privet, the leaf of Ivy (*Hedera Helix*) may be used: the structure is essentially the same, but the following differences may be noted. The palisade cells are square rather than oblong. glandular hairs are absent. sclerenchyma and occasional resin passages are found about the larger veins.

The Holly (*Ilex aquifolium*) may also be cut, but it is a xerophytic type. In sections treated with chlor-zinc-iodine, examine the **epidermis** under a high power: its outer wall is thicker than the rest, and is differentiated into —

a. **Cuticle**, a continuous, well-defined and highly-refractive layer, covering the whole epidermis externally. this stains yellow with chlor-zinc-iodine.

b. **Cuticularized layers**, of granular appearance, and stained a deeper colour than (a) with chlor-zinc-iodine; they are intermediate in properties between cuticle and true cellulose.

c. The **cellulose-layer**, which abuts on the cavities of the cells; this stains blue with chlor-zinc-iodine.

Note the presence of an additional layer of **hypoderma**, a strengthening tissue, which is immediately below the epidermis and consists of a single layer of cells at the midrib it may widen into two layers the walls are pitted, and stain bluish with chlor-zinc-iodine

V. **Included Starch-grains**.—These may be observed in the cells of the mesophyll of any leaf which has been exposed to the light, under conditions suitable for assimilation, or in the guard-cells of the stoma : but they may be seen with special ease in Fern prothalli which have been exposed to bright sunlight for some hours, and then bleached in alcohol

Mount the transverse section of a leaf, or a bleached prothallus in water, or in weak glycerine ; examine under a high power, and note the bleached chlorophyll-corpuscles, or **chloroplasts**, in which highly refractive granules may often be seen.

a Stain with iodine solution : the chloroplasts will assume a dusky bluish colour, the blue tint being more or less distinctly localized in the highly refractive granules (**starch-grains**) above noted

b. The presence of the included starch-grains may be more clearly demonstrated by causing them to swell : this may be effected in various ways.

- i. Mount in glycerine and iodine, and warm. the high temperature will swell the starch, which will at the same time stain with the iodine.
- ii. Treat with potash, and, after carefully washing out the alkali, stain with iodine.
- iii. The best method is, however, to treat the bleached specimens for some hours with a solution of iodine in chloral hydrate : the included starch-grains are simultaneously swollen and stained blue.

VI. **Water stomata and marginal glands**.—Examine leaves of the *Fuchsia*, and observe that the tips of the leaves and the marginal teeth are terminated by slightly swollen, opaque masses of tissue : these are the marginal glands, and drops of water exuded at those points may be seen in the morning, or on plants kept during the day in moist atmosphere at a high temperature.

Cut off a piece of the margin of a leaf and examine it under a low power a large stoma (**water-stoma**) may be recognized at the apex of each tooth below it is a pad of opaque tissue (the gland) to which a vascular bundle runs up, and in which it ends.

From material which has been hardened in alcohol, cut off with a razor the extreme tips of several of these teeth mount with the outer surface uppermost on examining these, the extreme apex, with the water-stoma will be seen in surface view Note the size of the stoma, and that the pore is widely expanded.

Cut longitudinal sections so as to follow the vascular bundle up to the marginal gland, and to traverse the gland in a median plane. Selecting a section which is really median, note, in the part of it further from the tip, the epidermis, mesophyll, and the vascular bundle surrounded by a parenchymatous sheath following these up towards the tip observe the epidermis which is continuous, with the exception of the widely-gaping water-stoma at the extreme apex the form of the guard-cells as seen in section is simpler than in ordinary stomata The vascular bundle widens out towards the tip, and the vascular elements terminate in the pad of closely-packed parenchyma of the gland (**epithema**) there is a large cavity below the water-stoma.

Special attention should be paid to the **chalk-glands** of the *Saxifragaceæ* (e.g. *Saxifraga crustata*) in which the structure of the marginal gland is extremely well seen, while the accretions of chalk deposited by the evaporating water are easily recognized with the naked eye. *S. oppositifolia* or *S. umbrosa* (London Pride) will afford excellent material for the study of these glands. Compare also various *Crassulaceæ*.

Treat some of the accretions with acetic acid, and note their solution with evolution of bubbles of CO_2 .

Leaf-scars and Fall of the Leaf.

On twigs of the Lime cut in winter, note the buds, both terminal and lateral, and below each an oval scar which indicates the surface of separation of a leaf when it fell in autumn

the surface of the scar is brown, and the slightly projecting dots upon it are the broken ends of the vascular bundles which ran out from the stem into the petiole.

Cut longitudinal sections so as to pass through a scar, and select for observation one of those which has followed up the course of one of the vascular bundles to the surface of the scar: mount in glycerine, and observe below the scar the tissues as above described for the stem (p. 67). At the level of the scar the following structural points are to be noted:—

1. The rough and irregular outer limit of the tissues, with dried up remains of cells often projecting beyond the general surface.

2. The dark brown band of **cork**, with phellogen on the inner side, continuous with that of the stem, traversing the leaf-base as a continuous protective sheet.

3. Lying outside the cork is a dried up mass of effete tissue. The outermost surface of this has been laid bare by the rupture of the **absciss-layer**.

4. The abstriction of the vascular bundle by invasion of parenchyma, capped by cork. also note that the vessels of the xylem are laterally compressed by the adjoining cells, and they are thus closed below the surface of the scar.

Sections should also be made from material taken in autumn just before the period of the fall of the leaf, so as to see the changes in the tissues at the base of the petiole which precede the rupture. In longitudinal sections through the base of such a leaf note the origin and position of the **absciss-layer**, where the rupture takes place: also that the tissues of the leaf above it are almost empty with the exception of crystals: those of the stem, below the layer of cork, have plentiful protoplasm and starch.

Comparisons may also be made of similar material of the Poplar, Ash, Walnut, or Horsechestnut, which are well adapted for illustrating the process of defoliation.

ROOT

Observations with the Naked Eye.

Germinate seeds of the Broad Bean or of the Pea, in coconut fibre or pure vegetable mould, at a moderate temperature, till the primary root has attained a length of six to eight inches

Note with the naked eye—

1. The **seed**, from which the testa can easily be removed, disclosing—

2. The two fleshy **cotyledons**: between these—

3. The **plumule**, which develops early as a stem, bearing foliage leaves.

4. Below the cotyledons a short **hypocotyledonary stem**, not clearly marked off externally, except by colour, from—

5. The **primary root**, on the upper part of which are—

6. Numerous secondary or **lateral roots**. These are formed in acropetal order, and are arranged in regular longitudinal rows, usually four in number. On the youngest part of the primary root no lateral roots are to be seen.

Observe that particles of the soil, &c., adhere to the older parts of the roots, while the younger apical parts come out of the soil quite clean: this is due to the fact that **root-hairs** are present on the older parts, but not on the younger parts close to the apex.

Microscopic Observations.

The sections may be cut from quite fresh roots, kept in water till the time of cutting, so as to be firm and resistant. or the material may be hardened in alcohol for some days. The root

may be supported by pieces of pith while cutting . mount some sections in glycerine, others in chlor-zinc-iodine.

1. Observe the following tissues, starting from the periphery —

1 The **pilliferous layer**, a single superficial layer. Single cells of this layer will be seen to have grown out perpendicularly to the surface as **root-hairs**, which as a rule are not branched, and are of cylindrical form, with thin cell-walls . particles of soil may be found attached to many of them

2 The parenchymatous **cortex**, a broad band of thin-walled tissue, with intercellular spaces, limited internally by—

3. The **endodermis** : this is a single layer of cells, having the characteristic **dark dot** on the radial walls. This is due to reflection of light from the peculiar sinuous waves of the central part of the radial walls . The oblique part of each wave acts as a reflector, so that the greater part of the light is diverted before it reaches the eye . hence the origin of the dark dot. Within this lies —

4. The **stele**, composed of the following parts .—

a. The **pericycle**, an undulating band of thin-walled cells, which is a single layer in thickness opposite the phloem, but opposite the xylem it consists of two to three layers of cells.

b. The **primary xylem**, consisting of radiating groups of elements, which are the most strongly marked tissues of the young root. The number of these is most often **four**, but it is subject to variation, and may be as high as six, while in the Pea it may frequently be only three. The groups of xylem have dark lignified walls (test with aniline sulphate), and resemble the primary xylem of the stem. Note that the smallest elements, the **protoxylem**, are at the periphery of each strand ; the xylem is therefore **exarch**, and its development **centripetal**.

c. An equal number of strands of **primary phloem** are found, alternating with the xylem-strands. These several groups of elements are separated laterally from one another by bands of—

d. **conjunctive parenchyma**, which also forms the thin-walled central **pith**.

II. Cut sections successively at older points in the same

roots, treat as before, and observe the mode of origin of the **lateral roots**, noting more especially the following facts .—

a. The lateral roots arise opposite the groups of primary xylem this explains their arrangement in four rows as above observed with the naked eye, since the number of groups of primary xylem is usually four.

b. The pericycle, by active meristematic division, gives rise to the lateral root.

c. The cells of the endodermis, and some of the cortex, also divide, giving rise to a **digestive sac**, outside the young root.

d. In the older lateral roots it may be seen that their vascular system is continuous with that of the main root.

e. The lateral roots, increasing in length, burst through the outer layers of cortex and the piliferous layer since they originate from deeply seated tissues and rupture the more superficial ones, they are said to be of endogenous origin.

The roots of Leguminosæ are liable to the formation of **root-tubercles**, which originate in a position similar to lateral roots, and are apt to be mistaken for them when young

III. As an alternative type of root *Ranunculus acris* may be taken : the roots are fibrous, and bear few lateral roots. Select one of the thickest, and from fresh, or spirit material, cut transverse sections at a point distant from the apex. Treat with caustic potash solution, and mount in glycerine. Observe successively the following tissues, starting from the periphery .—

1 The **piliferous layer**, of rather irregular cells, with but few root-hairs.

2 The **cortex**, the outermost layer of which directly within the piliferous layer is the **exodermis** ; its cells regularly alternate with those of the piliferous layer ; their walls are suberised with age, and the radial ones marked with bars. It is a specialized layer of the cortex, and is not present in all roots.

The rest of the **cortex** consists of a broad band of thin-walled parenchyma, with intercellular spaces ; it is often full of starch. Its innermost layer is .—

3. The **endodermis**, which shows when young the characteristic dot on the radial walls (see 3, on p. 94) : at a later stage

the cells lying opposite the phloem become thick-walled and lignified, while those opposite the xylem remain thin-walled.

4. The **stele**, consisting of—

a. The **pericycle**, which forms a single layer of thin-walled cells running round the periphery.

b. The **xylem**, consisting of three to five separate groups of elements radiating from the centre: at the outer point of each group lie the protoxylem elements, distinguished by their small size and early formation. The later-formed xylem elements, usually meet at the centre of the stele, although sometimes a small pith may be present

c. The **phloem**, consisting of a like number of groups alternating with those of the xylem. They have thin cellulose walls.

d. Cells of the **conjunctive parenchyma** intervene between the phloem and the xylem.

IV. Cut transverse sections of the root of the Pea or Bean, six inches or more from the apex, avoiding the lateral roots. take care also to avoid the thick base of the hypocotyledonary stem, which shows a structure characteristic neither of the stem nor of the root: treat as before.

The general arrangement of tissues is the same as has been above described, though there has been increase in bulk, and the xylem and phloem, being now more fully developed, are more easily recognized. Observe especially that the **conjunctive parenchyma**, lying centrally to the phloem, has begun to divide repeatedly by tangential walls. The activity thus initiated extends round the peripheral margin of each xylem-group. The meristematic ring thus completed is the source of the **secondary thickening** of the root.

V Cut transverse sections of an old root of the Pea, or preferably of the Bean, taking care here also to avoid the base of the hypocotyledonary stem, and treat as before. Observe—

1. Centrally a **parenchymatous pith**

2. The **primary xylem-groups**, usually four in number, retain their original position, relatively to the pith.

3. The four cambial arcs, inside of the phloem-groups, have produced four large wedges of **secondary xylem** on their inside, and **secondary phloem** on their outside.

4. Four broad **parenchymatous rays**, which lie on the same radii as the primary xylem, have resulted from cambial activity opposite the protoxylems.

5. The **primary phloem-groups**, now separated by the mass of secondary tissues from the primary xylem, are still on radii alternating with them.

6. A narrow band of **cork** with a **cork-cambium** at its inner limit; it originates from the pericycle, and this fact should be ascertained by cutting sections successively at younger and older points. It is to be noted that the endodermis, cortex, and piliferous layer, are absent in these sections, being thrown off on the formation of the layer of cork from the pericycle beneath them, which thus cuts them off from a physiological connection with the central cylinder (Compare Fig 7, p 98)

VI. The structure thus described for the Pea or Bean is characteristic for Herbaceous Dicotyledons. In Woody plants the process is essentially the same—it may be studied in the Sycamore, Horse-chestnut, &c. Dig up roots of these plants carefully so as not to break off the finer fibrils. wash them gently from the soil, and observe the reddish-brown colour of the thicker and more mature parts, while the ends of the thinner fibrils are pale-coloured. Note also on passing from young portions to the older that an outer coating of effete brown tissue is thrown off, and thereby the bulk of the root may be diminished, notwithstanding the secondary increase of vascular tissue. The tissue thrown off is the cortex, which here, as in other cases, is only a temporary covering of the younger portion of the root. The soil will be found in this case also adherent to the rootlets, thus indicating the presence of root-hairs at some distance from the apex, but not at the extreme apex, nor on the older portion of the root where the cortex has been thrown off.

In transverse sections of such roots as these, of successive ages, the general similarity of structure to that above described, may be verified, with variations such as the following.—

1. The presence of an **exodermis**, immediately below the piliferous layer.

2. Small size, or complete absence of a central pith.

3. The origin of **secondary medullary rays**, and of **annual**

rings in the xylem. So that an old root resembles a stem in structure, but it may always be distinguished by its **primary xylem groups**, which retain their original position near the centre.

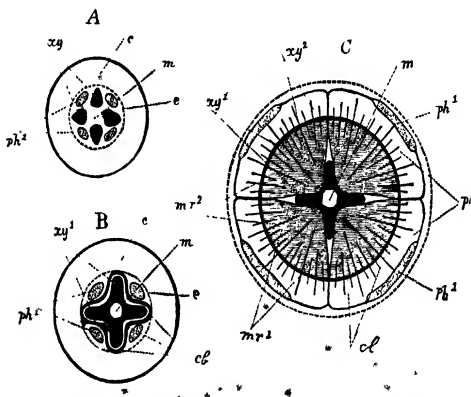


FIG. 4.—A. Diagram illustrating the disposition of tissues in the young root of a Dicotyledon before the cambial divisions begin.

B. The same at a later stage, when the cambium may be clearly recognized.

C. Diagram of arrangement of tissues in the root, after secondary thickening has been in progress for a considerable time. In such a root the cortex, which is seen as a broad band (c) in Figs. A, B, has been completely thrown off, and is not represented in the figure. c = cortex; e = endodermis; ph^1 = primary phloem; ph^2 = secondary phloem; xy^1 = primary xylem; xy^2 = secondary xylem; mr^1 = primary medullary rays; mr^2 = secondary medullary rays; m = pith.

Apex of the Root.

TYPE I.—Using the fruit of the Sunflower in the dry state, as it may be bought in seedsmen's shops, cut thin median longitudinal sections of the apex of the radicle of the straight embryo. The arrangement of the meristem at the apex of the radicle of

the embryo is similar to that of the apex of the growing root, and the former is chosen in this case, as it is much easier to make preparations from it than from the growing root. The sections are of little use unless they are accurately median.

Treat the sections with potash for ten minutes or more, or better, treat with "eau de javelle" as directed on p. 35 wash with water, and mount in glycerine. examine with a low power, and observe that—

1. The mass of tissue is composed of thin-walled cells, arranged regularly in longitudinal rows.

2. That these rows of cells converge towards a point at some distance below the external apex of the root. This is the centre of construction.

3. Note also that the vascular cylinder or stele of the root may be traced upwards to the apex as the **plerome cylinder**, with a dome-like ending.

4. The cortex continues upwards as the **periblem**: it may be distinguished by its darker appearance owing to the numerous intercellular spaces.

5. The superficial **piliferous layer**, which curves inwards under—

6. The tissue of the **root-cap**.

The general scheme of arrangement of the apical meristem of a root is indicated in the diagram, Fig. 8. but in comparing the sections with the diagram it must be remembered that the figure represents an ideal Dicotyledonous type, and it must not be attempted to trace a correspondence of minute detail of the sections with the diagram: thus in the diagram there is a sharp limit K K, between the root-cap and the body of the root, whereas in the Sunflower, as will be presently shown, the root-cap and piliferous layer have a common origin.

Examine with a high power: and observe that—

1. At some distance from the apex a definite **piliferous layer** covers the root externally. Follow this towards the apex: at some short distance from it this single layer splits into two: the inner is continuous over the apex as the **dermatogen**: the outer is one of the layers of the **calyptra**, or root-cap. Following the dermatogen further inwards, it will be seen to split

again several times in succession. The layers thus thrown off externally from the dermatogen form collectively the **root-cap**, or **calyptra**. We have in this case a common formative layer for root-cap and piliferous layer

2. Between the plerome cylinder and the piliferous layer lies a broad band of formative tissue of the cortex, or **periblem**: follow this to the apex it is also a distinct continuous band, though reduced to a single layer of cells at the apex.

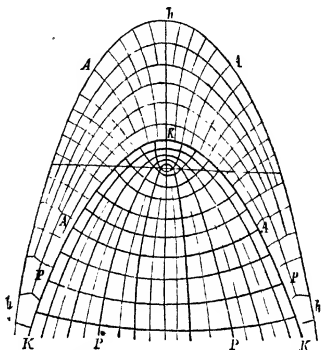


FIG. F.—Diagram illustrating the arrangement of a meristem which may be found at the apex of a root of a Dicotyledon. *h-h*, the root-cap, limited internally by the line *k-k*, which represents the outer limiting wall of the dermatogen. In the root-cap the letters *A-A* indicate anticlinals, *P-P*, the periclinal curves. (After Sachs.)

3. The **plerome** cylinder may also be traced as distinct up to the apical point.

To this type belong most of the Dicotyledons. The work may be equally well done on *Linum usitatissimum*, or *Polygonum Fagopyrum*.

TYPE II.—Prepare median longitudinal sections of the apex of the radicle of *Phaseolus multiflorus* (the Scarlet Runner), or

perhaps better, from the Broad Bean in either case the dry seed may be used, treat as the above. Examine with a low power and make out—

1. Calyptra (root-cap)
2. Piliferous layer.
3. Periblem.
4. Plerome

But here all the different tissue-systems will be found to originate from a general meristem, the original formative tissue of none of them being distinct from that of the others

As alternative plants of the same type, may be named *Cucurbita* and *Pisum*.

VEGETATIVE ORGANS —(B) MONOCOTYLEDONS

Observations with the naked eye

The grain of the Maize (*Zea Mays*) has been described on p. 40, and the germination on p. 44.

Fruits of Maize should be sown in pans, in spring, and kept in a cool house or frame: they may be bedded out in early summer, and the plants will be suitable for the observations detailed below in August.

In seedlings which have grown a foot or more in height observe—

1. The **green foliage leaves**, arranged alternately, with $\frac{1}{2}$ divergence upon—
2. The relatively short **stem**, which is continuous downwards with one short basal internode, to—
3. The main **root**, which is usually more strongly developed than the numerous adventitious roots.

Note the **grain** which may still be laterally attached, at the lower extremity of the basal internode; while from the upper extremity, bursting through the lowest leaf-sheath (the cotyledon of Hofmeister and others) are numerous **adventitious roots**. In older plants successive internodes show successive series of such roots, which form efficient supports for the obconical stem of the mature plant.

Examine a single leaf, and observe—

1. The expanded **lamina**, with parallel venation, and in old leaves the hairy upper surface, while the lower surface is not obviously hairy.
2. The **leaf-sheath**, enfolding the axis or next younger leaves.
3. The frill-like **ligule**, at the junction of sheath and lamina.

i. STEM—*HERBACEOUS TYPE*

Fresh material may be used, but stems, well matured in autumn, and preserved in alcohol, are preferable.

1. Cut transverse sections from about the middle of an internode of a well-grown stem of *Zea Mays* : mount in glycerine, others in chlor-zinc-iodine. The sections should be cut from the upper part of one of the lower internodes, otherwise the vascular bundles may be found to be imperfectly developed.

Examine with a low power, and, beginning the study of the tissues at the periphery of the section, observe—

1. A single layer of rather irregular, often sclerenchymatous **epidermis**, stomata are frequently found : immediately below this is —

2. A **cortex**, consisting of usually three or four layers of cells, more or less sclerenchymatous, with no definite internal limit

3. A **parenchymatous ground tissue**, which forms the matrix of the central part of the section : embedded in this are—

4. Numerous **vascular bundles** : note that they are smaller, but more numerous near the periphery than at the centre ; also that the position of the parts of the bundles relatively to the centre of the section is usually uniform

Examine in detail a section treated with chlor-zinc-iodine and observe —

a. The **epidermis** appears as a definite layer of cells of unequal size. Note a well-marked **cuticle** (brown). Here and there may be found **stomata**, with two small **guard-cells**, and two **subsidiary cells**.

b. The **cortex** consists for the most part of sclerenchymatous cells with thick, highly refractive walls, which stain yellowish brown with chlor-zinc-iodine (lignified) : but this varies according to age, and proximity to a node.

c. The **parenchyma** consists of cells with thin cellulose walls. At the angles where the cell-walls meet are intercellular spaces. The external layers have abundant **protoplasm** with chloro-

phell corpuscles. These are less frequent in the inner layers, while in the central parenchyma the protoplasm is hardly appreciable.

d. For the minute study of the **vascular bundles** select one of the largest central bundles. The section must be thin. The most prominent elements in the bundle are --

i. Four large **vessels** of the **xylem**, arranged like a **V**, with the angle toward the centre of the stem. Of these the two smaller are developed first and constitute the **protoxylem**. Compare section of young stem.

The vessel nearest the centre of the stem has usually **annular** thickening. In old stems it is partially surrounded by an inter-cellular space, while the rings often become detached, in which case the vessel is not easily seen in transverse sections. Next to this is a vessel which has commonly a **spiral** thickening. The remaining two have thinner walls with **pitted** marking, and large cavity.

Between the pitted vessels are

ii. A number of **tracheides** with pitted lignified walls, and no cell contents.

iii. Parenchymatous cells with thin cellulose walls, which surround the inter-cellular space above described, and also the vessels. These may be regarded as **xylem-parenchyma**.

The **phloem** portion of the bundle lies between the limits of the **V** shaped xylem, and is easily recognized by the thin cellulose walls. It consists of

iv. Elements with large cavities, in which transverse septa (sieve-plates) often occur. These elements are the **sieve-tubes**.

v. Smaller **companion cells** between the sieve-tubes. At the outside of the phloem lies

vi. The **protophloem**, with its elements crushed out of shape.

Surrounding the above tissues of the xylem and phloem is a **sheath of sclerenchyma**. On its internal side may be found tissue-forms which are transitional between sclerenchyma and certain constituents of the bundle. In the more central bundles the sheath may be interrupted on either side opposite the **phloem** by thinner walled cells.

II Cut radial longitudinal sections of the same, treat as before, and observe—

a The **epidermis**, composed of oblong cells

b The narrow **cortex**, consisting of cells, which are sometimes thin-walled and parenchymatous, sometimes thick-walled and prosenchymatous

c The **ground-parenchyma**, with roundish cells

d The **vascular bundles**, pursuing a longitudinal course parallel to one another, without lateral fusion

In the **xylem** observe—

i The **annular**, **spiral**, and **pitted** vessels, and note especially in the latter, the clearly-marked joints, pointing to their origin from a succession of cells

ii The pitted **tracheides**

iii The thin-walled **xylem-parenchyma**

And in the **phloem**, which is easily recognized by its cellulose walls, distinguish—

iv The **sieve-tubes**, which have a wide cavity, intercepted here and there by transverse **sieves**

If it be found difficult to distinguish the sieve-plates, a fresh section may be treated with potash; the character of the sieve-plate is then more easily seen

v The **cambiform cells**, which are narrow and parenchymatous

Note the prosenchymatous constituents of the sheath of **sclerenchyma**, and observe transitional forms between these and the pitted elements with square ends, which belong to the xylem

The stem of *Asparagus* may be used for the above observations. The chief differences will be in the presence of a broad band of cortex, with a definite endodermis; the absence of sclerenchyma-sheaths round the individual bundles; and the sclerotic nature of the periphery of the conjunctive tissue of the stele

III In order to see the fundamental arrangement of the vascular system, cut median longitudinal sections through the apex of a young plant of Maize, or of a foliage-branch of an old plant—treat with strong potash; or, better, with dilute potash

for twenty-four hours : examine with a low power, and observe, if the section be median—

1 The **apical cone**.

2. **Leaves**, in successive stages of development, seated laterally

3 In the older leaves, **vascular bundles**, which enter the stem

Follow carefully the **course of the vascular bundles** as they leave the leaf-base. On following the course of these vascular bundles it will be seen that they enter the stem, and proceed at first towards the centre before reaching it they curve downwards, and finally turn again outwards, and approach the periphery of the stem. We thus see that in young stems of Maize the course of the bundles corresponds to the Palm-type, though as the stem grows older, and the internodes develop, the correspondence is less obvious, by reason of the almost straight course pursued by the bundles in the internode, and the complications which arise at the node.

But no student should be satisfied with seeing the typical bundle-system of a Monocotyledon in small microscopic preparations for it is not difficult to prepare from any of the Palms which have a columnar stem, dissections which shall show plainly to the naked eye the course of the vascular bundles. The spiral curvature of the bundles in a tangential direction, as they pursue their downward course, may be readily recognized in such dissections, where the ground tissue has been removed to a sufficient depth. No botanic institution should be without such dissections, which will make more plain to the mind than any description, or any microscopic preparation, the rather complicated bundle-system of the Palm-type.

ii. STEM—*ARBOREOUS TYPE*

1. Examine preparations of the old stem of *Yucca* or *Dracæna*, in which the thin-walled parenchyma has been allowed to rot away, while the vascular bundles remain. On

comparing transverse and longitudinal sections of such stems, it may be seen, with the naked eye—

1. That the central **primary bundles** are isolated, and that the course of each bundle may be traced as starting from below at the periphery of the stem, then curving towards the centre as it ascends, and finally turning outwards, and passing into a leaf. These are therefore **leaf-trace bundles**.

2. Towards the base of the stem there is a peripheral mass of secondary bundles, which increases in thickness downwards. These bundles have no direct connection with the leaves, and are therefore **cauline**.

II. Cut transverse sections of the stem of *Dracæna* immediately below the apical tuft of leaves. Mount in glycerine, and examine under a low power. Observe that the arrangement of the tissues, as well as the character of the vascular bundles, is similar to that in the Maize, but the cortex of *Dracæna* is much broader than in the Maize. Note that the cells of the inner limit of the cortex are quiescent, and not undergoing division.

III. Cut transverse sections of the stem of *Dracæna* at a point one foot or more from the apex, and mount in glycerine. Examine with a low power, and observe—

1. A well-marked **epidermis**. Beneath this—

2. A band of **cork**.

3. A broad belt of **cortical parenchyma**, many cells of which contain crystals (raphides, &c.). Here and there a vascular bundle will be seen in the cortex: these are bundles of the leaf-trace, passing inwards from the leaves.

4. At the inner limit of this the cells are not quiescent, as in the younger part of the stem, but there is an actively dividing **merismatic ring**, which gives rise internally to new vascular bundles, and externally to fresh cortical cells. The new bundles thus formed are **cauline**, having no direct connection with the leaves, and are embedded in lignified ground-tissue. These together form a dense ring.

5. Centrally, there still remains undisturbed that arrangement of thin-walled **parenchyma** and **vascular bundles**, which has been above noted in the young stem as being similar to

that in the internode of Maize: the primary or common bundles may be distinguished from the secondary or cauline bundles, not only by their arrangement, but also by their structure in the latter the phloem forms a small central group, entirely surrounded by the xylem the former are of the type already described for the Maize.

III. STEM—AQUATIC TYPE

Cut transverse sections from the submerged stem of the American Water Weed (*Elodea canadensis*). Mount in glycerine, and observe in the small circular section —

1. A regular **epidermis**, without stomata.
2. A broad thin-walled **cortex**.
3. A small central **stele**, limited by a well-marked **endodermis**, and consisting entirely of thin-walled elements. The absence of lignified elements is to be correlated with its submerged habit. This is an extreme case of reduction of the vascular system in aquatic plants.

LEAF

The external characters of the Maize leaf have been noted above, p. 102.

I. Treat a piece of the thin peripheral part of a leaf (which has been previously bleached in alcohol) with potash until it is transparent, mount in glycerine, and examine under a low power. Observe—

1. The **parallel** course of the **vascular bundles**.
2. Their frequent **lateral fusion**, by means of small branch-bundles.
3. The absence of **stomata** above the vascular bundles, and their arrangement in rows in the spaces between them.
4. The various forms of **hair**; and especially the conical unicellular hairs, which give the ciliate character to the margin of the leaf.

II. Cut transverse sections of the lamina; mount in water or dilute glycerine. Other sections may be treated with alcohol to expel the air-bubbles; the chlorophyll will, at the same time, be dissolved out. Other sections may be mounted in chlor-zinc-iodine, and kept for comparison with the above.

The section presents a **sinuous outline**, corresponding to a certain extent to the arrangement of the main vascular bundles. At the midrib the section widens out. Note the following arrangement of tissues—

1. Covering both surfaces of the leaf is an **epidermis**, resembling that of the stem, but the upper epidermis bears **hairs** of various form, mostly simple, conical. The largest of them are surrounded at the base by an **outgrowth** of the neighbouring epidermal cells. Small hairs are found on the lower epidermis.

Note the **stomata** on both surfaces, with small **guard-cells**, surrounded by two **subsidiary cells**; these will be further examined below.

On the upper surface observe how the epidermis here and there widens out into groups of deeper cells, with **non-cuticularised** outer walls, while the outer walls of the rest of the epidermis are

strongly cuticularised. These cell-groups occur at the thinnest regions of the section.

2. **Vascular bundles** of various size, which, in the thinner part of the lamina, lie in a median position between the two epidermal layers. Each bundle is surrounded by a definite parenchymatous sheath. The largest of them correspond in structure to those of the internode, the smaller ones are reduced forms of the same type. Note that the spiral and annular vessels of the **protoxylem** are nearer the upper surface of the leaf.

Between the epidermis on either side, and the larger bundles, are masses of **sclerenchyma**, which, together with the bundles, form complete bridges of rigid tissue between the two epidermal layers.

3. The spaces between the tissues hitherto considered are filled with **parenchyma** (**mesophyll**), which may either be (a) green (containing chlorophyll), or (b) colourless (without chlorophyll).

a. The green chlorophyll-containing parenchyma fills up the greater part of the space, often showing a palisade arrangement; intercellular spaces occur in it, and especially large ones are found below the stomata.

b. The colourless parenchyma occurs (i) as a sheath, without intercellular spaces, surrounding each bundle; (ii.) as groups of cells immediately below the epidermis. these are more common towards the central part of the leaf. At the midrib this tissue forms the bulk of the structure.

III. Cut thin tangential sections from the under surface of the lamina, so as to remove, if possible, only the epidermis. Treat with potash, and mount in glycerine. Observe—

1. The ordinary cells of the **epidermis**, of oblong form, and with sinuous outline.

2. Very short cells between the ends of these, which often project perpendicularly to the surface as **hairs**.

3. The **stomata**, also intervening between the ends of the oblong epidermal cells.

Observe with a high power the structure of the stomata. They consist of—

a. Two narrow **guard-cells**, which inclose the pore. Note their dumb-bell shape, with deeper and wider ends, and slim middle region.

b. Two triangular **subsidiary-cells**, which flank the guard-cells.

Compare this view of the stoma with the same structure as seen in transverse sections of the lamina—the dumb-bell shape of the guard-cells will then be properly understood. This type of stoma is characteristic of Grasses.

IV. Cut tangential sections of the upper surface of the Maize leaf, and compare with the above. Note the stomata, as before, arranged in longitudinal rows, the cells adjoining them having sinuous walls. Hairs are found in this region.

Note longitudinal bands of square cells, with lateral walls not sinuous, and outer walls not cuticularised. These correspond to the groups of larger cells already noted in the upper epidermis in the transverse section. Hairs are absent from these bands.

V. For comparison with the above, cut transverse sections also of the leaf of *Deschampsia cespitosa*, which has a smooth and convex lower surface, and longitudinally grooved, concave upper surface.

Note in the section that the general construction is not unlike that of the Maize. But the upper surface shows a succession of deep **ridges**, and **furrows**—stomata are absent from the lower surface, but are numerous on the slopes of the upper surface. Each ridge is topped by a **sclerotic band**, opposed on the lower surface by a corresponding band, while a main vascular bundle lies between. At the base of each groove lies a group of large, non-cuticularised watery cells, similar to those observed on the upper surface of the Maize. In this case, by modification of the Maize type of leaf construction, we recognize a mechanism for automatic curling upward of the leaf-margins under dry conditions. Compare also leaves of *Elymus arenarius*, *Ammophila arundinacea*, and of *Festuca ovina*.

VI. Cut sections also of the leaf of *Phormium tenax*, New Zealand flax. Note the smooth surfaces of the thick leaf; the upper epidermis is strongly cuticularised, without stomata: several layers of colourless hypodermis follow. The stomata

are present on the thinner lower epidermis. The girder system of sclerenchyma is arranged with great regularity. Chlorophyll-containing mesophyll is disposed towards the upper and lower surfaces, while large thin-walled cells, without chlorophyll (aqueous tissue), occupy the middle region.

VII Transverse sections should also be cut from the foliage-leaves of the Hyacinth, which, as above noted, grow in an almost vertical position mount in chloro-zinc-iodine, and note under a low power that there is no great difference between the upper and lower surfaces as regards the disposition of the tissues, excepting that the orientation of the collateral bundles is such that the xylem is directed towards the upper surface.

Stomata may be seen both on the lower and upper surfaces. there is no distinctly marked palisade-parenchyma centrally is a mass of colourless thin-walled parenchyma. Note the absence of strengthening sclerenchyma

This will be found an excellent opportunity for the study of the **details of a simple stoma** observe that the guard-cells are about at the general level of the epidermis that when fairly cut through the middle they differ in section from the other epidermal cells

The opposing faces of the two guard-cells exactly resemble each other, and are so curved that the space between them has the outline of an hourglass, its median constriction being the actual **pore**. The chamber on either side of this pore is partially enclosed by a projecting ridge of the guard-cell wall the one may be called the **outer**, the other the **inner chamber**. The cell-wall separating the guard-cells from the adjoining epidermal cells is relatively thin, that bordering the stomatal passage is thick, except opposite the actual pore. The cuticle is seen to be continuous through the pore, to the lower surface of the epidermis. Note also the large air-chamber leading into the system of intercellular spaces of the cortex. The contents of the guard-cells include chlorophyll-grains, and owing to their activity, a blue starch reaction is usually obtained with chloro-zinc-iodine. The above observations may be equally well made in sections of the leaf of *Lilium*.

VIII. To observe the stoma in surface view, and in the living

condition, take leaves of Hyacinth, or better of some species of *Lilium* or *Narcissus*, in which the stomata are of unusually large size. On a bright day, and after full exposure to the light, strip off a piece of the epidermis. mount it in water, with the outer surface uppermost, and examine under a low power. It may then be readily seen that the **pores** of the stomata are **widely open**, the guard-cells being strongly curved.

Having seen this, irrigate with a 2 per cent solution of common salt, keeping watch upon a stoma which has been seen to be open. When the salt solution reaches it, the stoma will be seen to close, the guard-cells straightening themselves as their internal tension is relieved, and finally becoming plasmolysed. The connection between the opening of the stoma and the internal tension of the guard-cells is thus demonstrated.

ROOT

I Cut transverse sections of the root of the Maize, selecting a well-grown, underground piece of root mount in glycerine, and examine first under a low power, then under a high power. Starting from the periphery observe—

1. The superficial **piliferous layer**, consisting of small cells, many of which have grown out into long **root-hairs**.

2. Immediately below this is a protective layer of **exodermis**, with more or less cuticularised cell-walls.

3. The **cortical parenchyma**, a broad band, of which the peripheral part is often sclerenchymatous, and this is especially the case in the aerial prop-roots observe the regularity of arrangement of the inner part of the cortex, of which the innermost layer is to be recognized as—

4. The **endodermis**: the cell-walls of this layer are in old roots thickened on three sides, the outer wall remaining thin and the radial walls in young roots show the usual dark dotted appearance.

5. Within this is the **stele**, consisting of —

a. The **pericycle**, a layer of cells with walls thin when young but they may be lignified when old the series is interrupted opposite the xylem-masses, which abut directly on the endodermis. Around the periphery of the stele are disposed —

b. Numerous **xylem-groups**, recognized by their dark-looking lignified walls: the smaller **protoxylem** elements are at the extreme periphery, the strand is therefore exarch.

c. **Phloem-groups**, alternating with the xylem-groups: they may be recognized by their brighter, cellulose walls. Note the number of xylem and phloem groups may vary, and is often very large.

d. Centrally lies a bulky **pith**, in which may be seen one or more isolated vessels, surrounded by irregular groups of sclerenchyma.

There is no secondary thickening.

II. Sections may also be cut transversely from the root of *Hyacinthus orientalis*.

The structure is essentially similar to that of the Maize, but the cortex is thin-walled, and in older roots may be partially disorganized. The endodermis is not thickened, but exhibits the characteristic dark dot on its radial walls. The pericycle is not interrupted by the protoxylem-groups—no pith is present, since the xylem-strands extend inwards to the centre of the stele.

Apex of the Root

It is not easy to cut longitudinal sections of the apex of an ordinary fully developed root without embedding. The arrangement of the meristematic tissues may be satisfactorily seen in longitudinal sections of the apex of the young lateral roots, which are to be found growing horizontally out of the nodes of the Maize plants. Or, if fitting material for this be not at hand, longitudinal sections may be made of the radicle of the embryo.

Adopting one of the above methods, cut longitudinal median sections of the apex of the root. The section must be accurately longitudinal and median, *i.e.* the section must include the organic axis of the root, around which the several tissues are symmetrically arranged.

Treat the sections for about ten minutes with dilute potash, or, better, with "eau de javelle" (Appendix A), and mount in glycerine.

In a median section the following arrangement of tissues will be visible:—

1. A central mass of tissue, clearly defined laterally, and rounded off at its apex, which is at some distance below the external apex of the root: this is the **plerome-cylinder** or formative tissue of the **stele**. If this tissue be traced back into the older part of the root, it will be found that its central part is continuous with the parenchymatous pith, while its peripheral part develops into the vascular ring. Note rows of larger cells, which may be traced back as continuous with the vessels of the

xylem In the central portion of the **plerome** are intercellular spaces, which appear black in sections from fresh material, being filled with air.

2 Surrounding the **plerome** is a broad band of tissue with intercellular spaces, which appear as irregular black lines. This tissue is the **periblem**, which is the formative tissue of the cortex.

3. Outside this is a single layer of cells somewhat elongated radially, and with a thick outer wall. this is the **dermatogen**, or formative tissue of the piliferous layer

If the section be accurately median, it will be possible to trace (2) and (3) upwards, till, immediately above the apex of the **plerome**, they merge into a single layer of cells. thus the formative tissue, from which the piliferous layer and cortex are derived, is represented at the apex by a single initial layer of cells

4 Outside the **dermatogen**, at the apex of the root, will be found another formative tissue, the cells of which divide parallel to the surface of the **dermatogen**: this is the **calyptrogen**-layer, which is formative of the tissues of the **root-cap**. The latter appears as a mass of parenchyma, covering the whole apex of the root: the outer cells of it will be seen to be undergoing mucilaginous degeneration of the cell-walls.

If the section has been cut from the root dormant in the ripe fruit of Maize, the apex will be found sheathed by a broad band of tissue of the **coleorhiza**. an embryonic structure, which is not present in growing roots.

Note on Raphides.

1. Cut longitudinal sections of the scape or **leaves** of the Hyacinth or Onion: many other Monocotyledons will do as well: mount in water, and observe the large cells containing numerous needle-shaped crystals (**Raphides**) arranged in a bundle parallel to one another. In order to investigate their nature the following tests may be applied:

a. The attempt may be made to stain them with iodine,

or other stains which colour crystalloids, but they will not be affected · they are thus distinguished at once from crystalloids

b. Irrigate a section with acetic acid · they are not affected : they are therefore not calcium carbonate.

c Irrigate with dilute nitric acid the crystals are dissolved They consist of calcium oxalate, and this form of crystal is common in Monocotyledons, and occurs also in some Dicotyledons. The crystals are often embedded in mucilage.

REPRODUCTIVE ORGANS

Observations with the Naked Eye on the Mature Flower

In order to become acquainted with the external characters of the reproductive organs, it will be well to examine and compare a few common types of flower.

1. Examine specimens of the common Buttercup (*Ranunculus acris*) a number of **flowers** may be found associated together on a single branching system, the **inflorescence**, which has here

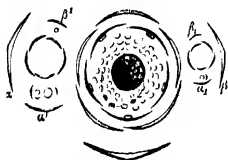


FIG. 9 -- Diagram of inflorescence and flower of *Ranunculus acris* (After Eichler)

the character of a cymose panicle. Recognize in each single flower the following series of parts, which are inserted upon the enlarged apex of the axis, or **floral receptacle** --

1. The **calyx**, which is the outermost series of floral leaves, and consists of five **sepals**, separate from one another (or **polysepalous**), inserted below the other organs (**inferior**), greenish yellow, and hairy

2. The **corolla**, consisting usually of five yellow **petals**, separate from one another (**polypetalous**), seated below the more central organs (**hypogynous**), and alternating with the sepals remove a single petal, and observe the pocket-like **gland** or **nectary** on the upper surface, close to the base.

3. The **androecium**, consisting of an indefinite number of **stamens**, which are separate from one another (**polyandrous**), and are seated below the central series of organs (**hypogynous**) :

each stamen is a club-shaped body, and two parts of it are to be recognized—

(a) A thin stalk, the **filament**.

(b) A two-lobed head, the **anther**.

In a flower fully opened, note with a lens the successive dehiscence of the anthers, by a longitudinal slit on either side of each, through which the powdery yellow **pollen** may escape. The outermost stamens dehisce first.

4 The **gynoecium** at the centre of the flower, consisting of an indefinite number of **carpels**, which are separate from one another (**apocarpous**), and are seated above the other floral organs on the conical receptacle (**superior**). Each carpel consists of a lower laterally compressed portion, the **ovary**, and at the apex of an upper, shortly curved process (the **style**) is a rough yellow surface, the **stigma**. Open one of the carpels carefully, and observe an internal cavity, containing a single round body, the **ovule**.

This is an **hermaphrodite** flower, with both male and female organs, of radial type (**actinomorphic**), and partially **protandrous**, the outer stamens being ripe before the stigmas are receptive, and thus it is liable to cross- or self-pollination.

II Compare the flower of *Caltha palustris*, the Marsh Marigold. Here the general arrangement of parts will be found to be the same—but note the following points of difference—that the calyx is here petaloid, consisting of five or more sepals, the corolla is absent, and the carpels are fewer (five to ten), but of larger size. Slit open one of the carpels along the dorsal side, turn back the flaps, and observe the **numerous ovules**—they are attached to the ventral side of the carpel, and are arranged in two longitudinal rows along the two margins of the folded carpel,—a **follicle** with **marginal placentation**.

III Compare the Monkshood (*Aconitum N. bellus*). the inflorescence is a long raceme, panicled at the base. The flower bears two **bracteoles**, right and left, on the pedicel. It is symmetrical only in an antero-posterior plane (**sygomorphic**), the posterior side being most strongly developed. The sepals are five, polysepalous, inferior, and **petaloid**, the posterior sepal developed as a large hood; the petals, usually eight, of which

six are small, the two posterior are elongated into glandular spurs (**nectaries**), and are covered by the hooded sepal. Stamens, spirally arranged, numerous and variable in number, polyandrous, hypogynous. Carpels usually three, apocarpous, superior follicles, similar to those of *Caltha*.



FIG. 10.—Floral diagram of *Aconitum Napellus*. *P* are the rudimentary petals. (After Eichler)

This is an example of the zygomorphic form, which ensures the visiting insect taking a definite position with regard to the essential organs of the flower. Note that the flower is **protandrous**.

IV. Dissect a flower from the simple raceme of the Wallflower (*Cheiranthus Cheiri*) note the absence of a bract subtending the pedicel. The flower consists of .—

1 **Calyx**, sepals four, **polysepalous**, inferior, the outer pair median, the inner transverse, with saccate bases.

2. **Corolla**, petals four, diagonally placed, **polypetalous**, hypogynous

3. **Andræcium**, stamens six, tetradynamous, *i.e.* four long (two antero-posterior pairs), and two short (transverse); nectaries are present at the base of the transverse stamens, which occupy the saccate bases of the transverse sepals

4 **Gynæcium**, carpels two, united (**syncarpous**), consisting of elongated ovary, short style, and bifid stigma.

Ovary bilocular (with "false" septum), placentation parietal, stigma-lobes directly above the placentas

Open the ovary carefully and note the parietal placentation, and the ovules of curved form (campylotropous).

This flower is hermaphrodite, homogamous, *i.e.* stamens and stigmas ripen at the same time; self pollination is possible, but colour, scent, and honey attract

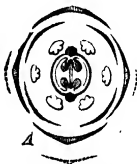


FIG. 11.—Floral diagram of an ordinary flower of the Cruciferae (After Eichler)

insects. Note the **cohesion** of the carpels, while all the other parts are free from one another.

V. Examine the greater Stitchwort (*Stellaria Holostea*), and note the regular cymose inflorescence (dichasium). The flower is composed of—

1. **Calyx**, sepals five, polysepalous, inferior.
2. **Corolla**, petals five, deeply notched, polypetalous, hypogynous
3. **Androecium**, stamens ten, polyandrous, or very slightly united at the base, hypogynous.
4. **Gynoecium**, carpels three, united or syncarpous, superior styles and stigmas three. Ovary unilocular, ovules numerous, inserted on the prolongation of the axis (**central placentation**)

VI. Compare the Rose Campon (*Lychnis dioica*), which belongs to the same natural order, and shows the same general characters, but the plants are more or less distinctly **unisexual**, flowers with perfect stamens being borne on some plants, while on others flowers will be found with only the female organs matured the species is thus **dioecious**, and a cross is therefore necessary

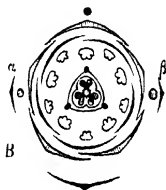


FIG. 12 — Floral diagram of *Silene inflata* (After Eichler.)

Note the calyx with united sepals, gamosepalous in the male flowers there are ten stamens slightly united at the base, and centrally a **rudimentary gynoecium**, in the female flowers, the gynoecium consists of five carpels, syncarpous and superior, while around its base may be seen ten **rudimentary stamens** thus this plant illustrates **cohesion** of the sepals and of the carpels, and a partial **suppression** of the stamens, or of the carpels.

VII. From a raceme of *Cytisus Laburnum* remove a single flower: observe its zygomorphic form, and note the following parts of it:—

Calyx two lipped, typically of five sepals, gamosepalous.

PRACTICAL BOTANY

Corolla petals five, polypetalous, consisting of a large posterior **vexillum**, lateral **alae**, and two anterior petals coherent together to form the **carina**, which envelops the essential parts.

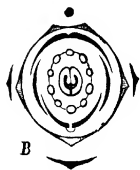


FIG. 13—Floral diagram of *Citrus Laburnum* (After Eichler)

Andrœcium, stamens ten, united by their filaments (**monadelphous**), inserted on the receptacle, slightly enlarged as a "calyx-tube" (**perigynous**).

Gynœcium, one carpel, superior open it and note the parietal placentation of the numerous campylotropous ovules

The flower is zygomorphic, and slightly protandrous there is no nice honey secretion, but a succulent swelling at the insertion of the vexillum.

VIII. Examine flowers of the Bird-Cherry (*Prunus Padus*) they are arranged in a racemose manner Note that each flower consists of—

1. A **calyx** of five sepals, inserted upon the so called calyx-tube, which may be regarded as an enlargement of the floral receptacle

2. A **corolla** of five polypetalous petals, also inserted on the margin of the calyx-tube, the petals alternating in position with the sepals.

- 3 **Andrœcium**, composed of indefinite stamens, polyandrous, and perigynous, i.e. inserted upon the calyx-tube

- 4 **Gynœcium**, consisting of one carpel, superior ovules two

This is a typical perigynous flower, in which the sepals, petals, and stamens are inserted on the calyx-tube, or cup-like receptacle.

IX Compare the Water Avens (*Geum rivale*), which is similarly constructed, but note the five small segments alternating with the sepal (**epicalyx**); the numerous stamens, showing clearly the perigynous insertion on the enlarged receptacular cup; the elongated



FIG. 14—Floral diagram of *Prunus Padus* (After Eichler)

stalk (**gynophore**) on which are borne upwards, the very numerous apocarpous carpels. Examine one carpel and note the one-ovuled ovary below, and the elongated hairy style, with a kink about one-third up, and the terminal stigma.

X Compare flowers of Hawthorn (*Crataegus oxyacantha*), which belongs to the same natural order: the disposition of the parts is the same as in the above. The carpels may vary in number. It differs however in the fact that from an early period the calyx-tube is **adherent** to the ovary, and the ovary accordingly is **inferior**. The apple (*Pyrus Malus*) may also be compared: the number of carpels is regularly five.

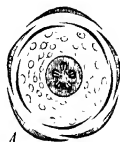


FIG. 15.—Floral diagram of *Mespilus germanica* (After Eichler)

XI From the large compound umbel of *Heracleum giganteum*, separate a single complete flower, and note its component parts:

Calyx, sepals five minute green teeth

Corolla petals five, polypetalous, alternating with the sepals, the peripheral one sometimes largest

Andræcium, five free stamens, alternating with petals

Gynæcium carpels two, antero-posterior; stigmas two rising from the glandular disc; ovary inferior, bilocular: dissect out the solitary pendulous ovule from each loculus



FIG. 16.—Floral diagram of *Eryngium maritimum* (After Eichler)

Note that there is a partial separation of the sexes in space, some flowers being hermaphrodite, while others are male, with abortive gynæcium: there is also a separation in time, the hermaphrodite flowers being protandrous.

XII Compare *Astrantia major*, with its simple umbel, and showy involucre of bracts. The floral construction is similar to the above, but the calyx-teeth are larger, the petals curved inwards, the stamens, incurved when young, open in succession; the hermaphrodite flowers are protan-

drous, and many of the flowers male by abortion of the gynoecium.

The honey in these flowers is exposed on the glandular disc, and is therefore accessible to short tongued insects.

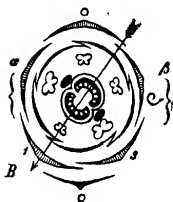


FIG. 17.—Floral diagram of *Petunia nyctaginiflora*. (After Eichler)

XIII. Examine the flower of the potato (*Solanum tuberosum*), it consists of —

Calyx, sepals five gamosepalous, inferior.

Corolla, petals five, gamopetalous, hypogynous, alternating with the sepals.

Androecium, stamens five, epipetalous, alternating with the petals, dehiscent by terminal pores

Gynoecium, carpels two, syncarpous, superior, oblique to the median plane of the flower style elongated, stigma slightly bifid ovary bilocular, ovules numerous, placentation axile.

XIV. Examine a single flower from the Cymose panicle of the Figwort (*Scrophularia nodosa*) it is zygomorphic and consists of the following parts —

Calyx, sepals five, gamosepalous, inferior, odd sepal posterior

Corolla, petals five, gamopetalous, odd petal anterior.

Androecium, stamens four, didynamous, epipetalous. but note a staminode, corresponding in position to the odd posterior stamen, here non-functional.

Gynoecium, carpels two, syncarpous, superior : ovary bilocular, ovules numerous, placentation axile. A yellow nectary surrounds the base of the ovary.

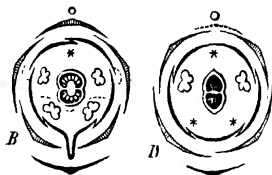


FIG. 18.—B, Floral diagram of *Linaria vulgaris* ; D, of *Veronica Chamadrys*. (After Eichler)

Note the **protogynous** condition, in flowers just opened the stigma is exposed, while the stamens are hidden within the corolla; in older flowers the stamens are exposed and dehiscent, while the stigma, if pollinated, is curved downwards.

Compare the flowers of *Linaria*, *Antirrhinum*, *Digitalis*, and *Mimulus*, in which the plan of construction is the same, but details of pollination mechanism different.

Dissect the flower of some species of *Veronica*, e.g. *V. Chamædrys* and compare it with that of other Scrophulariaceæ. Note the calyx sepals four, by abortion of the posterior segment: corolla rotate, with four lobes, of which the largest is the result of fusion of two obliquely posterior petals. Stamens two, occupying the place of the posterior-lateral stamens of other Scrophulariaceæ, the other three being suppressed.

XV. From the axillary verticillate cymes of the White Dead Nettle (*Lamium album*), separate a single flower, and observe as follows —

Calyx, five sepals gamosepalous, inferior the odd sepal posterior.

Corolla, petals five, gamopetalous hypogynous, the lower part is tubular, the upper two-lipped the upper lip is composed of two petals, forming a hood covering the essential parts the other three petals constitute the lower lip

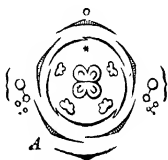


FIG. 19 — Floral diagram of *Lamium album* (After Eichler)

Androecium, stamens four, didynamous epipetalous, opening downwards.

Gynœcium, carpels two, syncarpous superior style filiform, stigma bifid. ovary spuriously quadrilocular, developed as four nutlets at the base of the ovary; on the anterior side is a fleshy nectary.

Note the absence, by abortion, of a fifth posterior stamen: also that the zygomorphic flower is homogamous, male and female organs being mature at the same time.

XVI. Examine flowers of the Primrose (*Primula vulgaris*), or of the hothouse Primula (*P. sinensis*), and note that it is composed of—

1. **Calyx** of five sepals, gamosepalous, inferior.
2. **Corolla** of five petals, gamopetalous, hypogynous, and alternating with the sepals

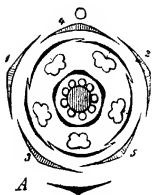


FIG. 20.—Floral diagram of *Primula acaulis*. (After Eichler.)

3 **Andrœcium** of five stamens, epipetalous—they are opposite the petals (**antipetalous**).

4 **Gynœcium** of five carpels, syncarpous, superior placentation, free-central, ovules numerous

This is an example of **cohesion** of sepals, petals, and carpels, and of **adhesion** of the stamens to the corolla. Note also that the flowers are of two types, on distinct plants (**dimorphic**). On some plants the flowers have long stamens and a short style, on others they have short stamens and a long style—they are

homogamous, but the dimorphic state increases the probability of intercrossing.

XVII. Examine a head or capitulum of the Sunflower: this is an inflorescence, and it is composed of a large number of florets, or small flowers, inserted on a wide disk-like development of the main axis or peduncle. Note the dark green, closely imbricated **bracts**, which show a gradual transition from the foliage-leaves to a simpler form: these together constitute the **involucre**, which surrounds the margin of the capitulum. On the upper surface of the flattened receptacle are the numerous, and closely packed **florets**, of which two types are to be distinguished—

a **Ligulate** or **ray-florets**, with broadly strap-shaped yellow corolla, which are disposed at the **periphery**.

b. **Tubular** or **disk-florets**, which constitute the central part of the head.

In the young inflorescence before flowering, and also later it



FIG. 21.—Diagram of tubular floret of a Composite: the pappus after *Carduus crispus* (After Eichler.)

the **fruiting** inflorescence, there may be seen opposite, and external to each floret a small leaf (the **bract**), lanceolate above, but broadly sheathing below, in the axil of which the floret is produced.

Remove the bracts from the periphery of the capitulum, and separate a single **ligulate floret**: examine it in detail, and observe at the base the more or less compressed **ovary**, which is inferior: at its upper limit is an irregular rim, which may be regarded as representing the **calyx**: above it is the yellow **corolla**, tubular in its lower part, broadly ligulate above—on slitting the tubular portion there may be seen a more or less reduced style inserted on the apex of the ovary—the stamens are abortive. These florets are thus neuter.

Examine one of the **florets of the disk** in detail, noting first its position in the axil of a bract—observe—

1. At the base the laterally compressed **ovary**, which is **inferior**.
2. Seated in an antero-posterior position above the ovary are two chaffy scales—these represent the **calyx**, which is accordingly **superior**.

3. Above this is the tubular yellow **corolla**, narrowed below, and terminated above by five teeth: it thus consists of five petals, gamopetalous and superior.

4. Projecting from the tube of the corolla may be seen a dark-coloured, cylindrical body composed of the coherent **anthers**, and projecting through this cylinder is—

5. The bifid and recurved **stigma**.

Slit the tube of the corolla longitudinally, and note the five separate filaments of the **stamens**, which insert themselves on the inner surface of the narrow throat of the corolla (**epipetalous**), while the anthers are united above so as to form a tube (**syngenesious**).

Remove the corolla and stamens: the long cylindrical **style** will remain rising from the apex of the ovary, and terminating in a **bifid stigma**: thus it is indicated that there are two carpels, syncarpous and inferior.

Open the ovary longitudinally, and note the single cavity (unilocular), and within it a single **anatropous** ovule attached to the base of the cavity.

As a substitute, or for comparison, the head or capitulum of the Dandelion (*Taraxacum officinale*) should also be dissected : the general features will be similar to the above, the chief points of difference being that—

1. All the florets are ligulate, and bisexual
2. The bracts on the receptacle are abortive.
3. The calyx is developed as a silky pappus.

The Ox-eye Daisy (*Chrysanthemum leucanthemum*) may also be examined : the general floral construction is the same as in the Sunflower, but there are no bracts on the receptacle, and no representative of the calyx.

XVIII. In any of the ordinary native species of Spurge (*Euphorbia*) note the following structure of the small contracted

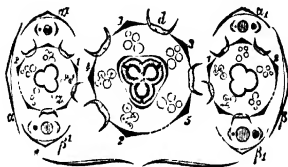


FIG 22.—Ground plan of dichasial branch of *Euphorbia Peplus* $\alpha\beta, \alpha'\beta'$ are the bracts, 1-5 the involucre leaves, in their genetic sequence d , glands in the indentations of the involucre. The three-lobed perianth on the \varnothing flower of the central cyathium is figured after certain exotic species in which it is clearly developed. (After Eichler)

flower-like inflorescence—the **cyathium** : these are commonly borne on a cymose, unbellate branch-system, with leafy bracts. The cyathium consists of a cup-like **involucre**, formed by five coherent bracts, between which are four large glands, the fifth space being vacant. Within the cup, the most prominent object is the **female flower**, which occupies the centre : it is long-stalked, and projects, curving laterally, over that side of the cup where there is no gland. Note its cylindrical stalk, terminating above in the **gynoeceum**, at the base of which is a distended ring, representing the **perianth**. Carpels three, syncarpous superior : styles three, with bifid stigmas : ovary trilocular,

ovules one in each loculus, pendulous anatropous. The **male flowers**, which are associated with minute hairy bracts, consist each of a **single stamen**—note half down the filament a joint—this is believed to represent an abortive perianth.

This is regarded as an extreme case of reduction of floral structure in a condensed inflorescence.

XIX. Examine male and female catkins of the common Sallow (*Salix caprea*)—they are borne on separate plants which are thus male or female (dioecious): the catkin, whether male or female, is a spicate inflorescence of numerous simple flowers, which are borne in the axils of bracts. Separate off one bract, with its appendages, which constitute the **male flower**: there are two stamens, right and left of the median plane, filaments free. note the gland at their base, on the side next the axis.

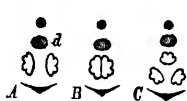


FIG. 23.—A, *Salix Caprea*; B, *S. purpurea*; C, *S. triandra*. Diagram of male flowers (After Eichler)

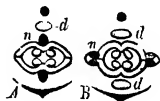


FIG. 24.—A *Salix Caprea*; B, *S. alba*. Diagram of female flowers (After Eichler)

The **female flower** consists of a gynoecium of two carpels, syncarpous, superior, in the transverse plane: ovary stalked, unilocular, ovules numerous. note the gland at the base, on the side next the axis.

The flowers of the Willows are probably primitively simple. The honey glands and scent attract numerous insects, while the dioecism necessitates intercrossing, which is carried out by them. Wind may also be efficacious.

XX. For comparison with the above types of flower, which are all from Dicotyledonous plants, examine, as typical of the Monocotyledons, the flowers of the wild Hyacinth (*Scilla nutans*), which are borne in simple racemes. Each is composed of—

1. A **perianth**, consisting of six petaloid segments which are free, or polypetalous, and hypogynous: three composing an

outer whorl, overlap the other three which compose an inner whorl.

2. The **androecium**, consisting of six stamens, each being opposite one of the segments of the perianth to which it adheres.



FIG. 25.—Floral diagram of *Ornithogalum umbellatum* (After Eichler)

- 3 The **gynœcium**, consisting of three carpels, syncarpous and superior the ovary has three loculi, ovules numerous, anatropous, placentation axile.

Other examples of flowers of the Liliaceæ may be compared, e.g. *Lilium*, *Convallaria*, &c. Compare *Galanthus*, or other example of the Amaryllidaceæ, in which the ovary is inferior, but the floral construction otherwise the same.

XXI. Examine the spicate inflorescence of *Orchis maculata*,

note the zygomorphic flowers, sessile in the axils of long bracts, and that the green inferior ovary of each is spirally twisted, so that the flower is turned through half a circle the anterior side has thus a posterior position, and the flower is **resupinate**. The flower is composed of the following parts —

Perianth, six segments, polypetalous, the odd posterior segment (anterior by resupination), enlarged as the **labellum**, and spurred

Androecium, only the outer anterior stamen developed (posterior by resupination). It is bilocular, and contains two pollen-masses (pollinia). The stamen is confluent with the style to form the short **column**.

Gynœcium, carpels three syncarpous; ovary inferior, twisted, unilocular; placentation parietal, ovules, minute, very numerous

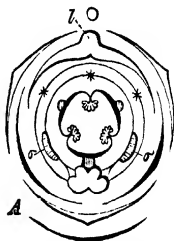


FIG. 26.—Floral diagram of *Orchis*. (After Eichler)

Observe the large semilunar surface of the stigma between the stamen and the entrance to the spur, this corresponds to the two obliquely posterior lobes of the stigma immediately above this is a projecting beak, the **rostellum**, which corresponds to the anterior stigmatic lobe, and covers the adhesive discs of the two pollinia

Insert a pencil point gently into the spur, in doing so, the rostellum is pushed aside, and the adhesive discs fixing to the surface of the pencil, the two pollinia are drawn out as it is withdrawn. Observe them carefully for a minute or so, and note their change of position, from the erect to a position parallel to the pencil point. On inserting the pencil into another flower, the pollinia will now come in contact with the stigmatic surface.

This is a complex mechanism leading to cross-pollination as a consequence of insect visits. without insect-agency pollination does not take place

XXII. Examine the inflorescence of *Lolium perenne*, it is a compound spike, bearing lateral spikelets alternately on opposite sides of the main axis

Separate off one spikelet complete, and recognize the following parts, starting from below at the base, and subtending the whole spikelet, is one empty **glume**, a green bract. In most Grasses another empty glume is found on the opposite side of the spikelet, but in *Lolium* this is absent. The rest of the spikelet is composed of successive pairs of bracts, **paleæ**, these pairs are inserted alternately on opposite sides of the thin, woody rachis of the spikelet. Separate one of the closely-folding pairs of paleæ from one another, between them will be found the **flower**, which is entirely enclosed by the bracts (paleæ), except at the period of flowering, when the three stamens and two stigmas are exerted. Having found a flower in this condition, note the following parts of it --

The **androecium**, consisting of three stamens, the **mid** stamen being anterior. They have long thin filaments, and anthers with dusty pollen.

The **gynoecium**, superior, with two lateral feathery stigmas, which project right and left from the paleæ. ovary unilocular, ovule solitary. At the base of the ovary on the anterior side are

two **lodicules**, which are important in forcing the paleæ apart, at the time of flowering

The structure of the spikelet is the same as this in plan in most Grasses. Some regard the flower as a reduction of the

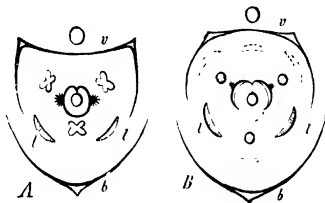


FIG. 27—Empirical (A) and theoretical (B) diagrams of an ordinary Grass-flower, according to the usual explanation (After Eichler)

Liliaceous type (cf. Fig 27, B), others do not accept this view (See Morphological Text-books)

For further details as to the various structure and form of flowers, and for discussions as to their morphological interpretation and pollination mechanisms, reference must be made to books on Descriptive Botany.

DEVELOPMENT OF THE FLOWER.

In order to trace the development of the flower it is found convenient to use plants with **aggregated inflorescences**, *i.e.* those in which the flowers are closely associated together in large numbers. By cutting sections through such an inflorescence many individual flowers, illustrating different degrees of progress, will be traversed, and by comparison of these an idea of the course of development may be gained even from a single section.

I. Examine young **capitula** of the Sunflower with the naked

eye they occur in the same positions as the vegetative apical buds, but differ externally from these—

- 1 In their greater bulk, and more especially in their diameter being larger

2. In their colour, which is usually darker

- 3 In being covered externally by a large number of imbricated **bracts**, which together form the **general involucre**

Select a very young capitulum— that is, one in which these characters can be recognized, but are not as yet very pronounced—and, having removed the largest external bracts, cut from it median longitudinal sections treat with potash and mount in glycerine observe with a low power—

- 1 That in outline and general arrangement of parts the sections resemble those of the vegetative bud, but that the apical cone is broader, and more flat

- 2 That the surface of the cone has an irregular outline, owing to the formation of a series of appendicular organs, which are developed in **acropetal order**, *i.e.* the smallest or youngest are nearest the centre or apex, while on passing towards the periphery the size regularly increases.

Put on a higher power, and study these organs in detail, beginning at the centre.

If the capitulum be young enough, there will be found, as in the vegetative bud, a naked **apical cone**, rather flatter in form, but with a similar arrangement of tissues to that there observed. Passing from the centre, the external surface assumes an undulating appearance owing to the formation of—

1. **Bracts**, or small leaves, which arise similarly to the leaves as above observed (p. 58), by outgrowth of the epidermis and subjacent tissue; as they grow older they curve towards the centre. Note the formation of **hairs** of various types from single cells of the epidermis, this being a good opportunity for tracing their origin.

2. The rudiments of **flowers**, which appear in the **axils** of the bracteoles, *i.e.* on the side nearer the apex. These are likewise produced from the epidermis and subjacent tissue; they are, morphologically speaking, **axillary branches**.

The development of the latter into the complete flower must

be carefully studied, by comparison of those nearer the centre with older flowers near the periphery of the capitulum, or on capitula of various ages. It is obvious that flowers which have been cut in median section will be best fitted for this study. Note the following successive stages of development—

a Form of papilla, conical

b. Apex becomes flattened.

c Periphery of the flattened apex rises into a whorl of five small lobes; these are the **petals**, which are in the mature flower united as a gamopetalous **corolla**.

d Between the corolla and the now depressed apex rises a fresh series or whorl of five lobes, these are the young **stamens**.

About this stage may be seen externally, below the corolla, a slight protuberance on each side of the flower (as seen in section) this is the first appearance of the **calyx**, which consists in the mature flower of two scaly sepals. This order of appearance of the floral whorl is not normal, but is the rule in the order *Compositae*. In the large majority of plants the calyx is developed first, then the corolla, and then the stamens.

e Within the whorl of stamens there arises, at the margin of the now much depressed apex, the last series of floral organs, viz two **carpels**, which arch over the apical depression, and thus close in the cavity of the **inferior ovary**.

f. All the organs increase in size, while from the base of the cavity of the ovary a papilla arises, which develops into a single **anatropous ovule**, with one **integument**, and small **nucellus**.

II. Cut horizontal (*i.e.* transverse) sections of a capitulum treat as before examine with a low power

Note the arrangement of bracteoles, with young flowers in their axils, round the central naked apex. The youngest flowers will appear simply circular in outline (simple papillæ of stages *a* and *b*); older flowers will show successively—

i. The five papillæ of the **corolla** (petals) uniting at an early stage into the gamopetalous corolla-tube. (Stage *c*.)

ii. Five **stamens**, alternating with the petals. (Stage *d*.)

iii. Centrally two **carpels**. (Stage *e*.)

Other *Compositæ* may be taken as substitutes for *Helianthus*, but some variety will be found in different genera in the character of the bracts, and in the calyx thus in *Chrysanthemum Leucanthemum*, which is a useful type, the bract subtending each flower is absent, and there is no representative of the calyx.

CALYX AND COROLLA

I. Mount in glycerine two small pieces of the corolla of a ligulate floret of the Sunflower which has been kept in spirit, the one with the lower, the other with the upper surface uppermost. Examine under a low power, and note the delicate texture and transparency of the whole the rarity or even entire absence of stomata the numerous hairs on the lower, and the small projecting papillæ on the upper surface and the vascular bundles which do not form a dense network as in the foliage leaf.

II. Cut transverse sections of the same, mount in glycerine, and observe that the structure is altogether simpler than that of the foliage leaf. Note the smooth lower epidermis the upper epidermis, of which each cell has grown out as a **conical papilla**: it is these which give the velvet-like appearance to the corolla the mesophyll which is very lax, and is not differentiated into palisade and spongy parenchyma.

The bright colours of flowers and fruits may be due either to colouring matter dissolved in the cell-sap, or to small coloured bodies in the cells, or to both combined. They must be studied in fresh material, as the colourings are altered or destroyed by alcohol. As a first example the common scarlet *Geranium Pelargonium*) may be taken, in which the petals owe their colour to dissolved matter.

III. Strip off the superficial tissue from a petal of this plant, and mount in water with the outer surface uppermost note under a low power the conical form of the superficial cells, and the **bright red colour of the cell-sap**: chromoplasts or formed granules appear to be entirely absent.

IV. The case of the common red and yellow Tulip is a good

example of mixed colouring ; strip off the superficial tissue from the yellow base of one of the segments of the perianth mount in water, and examine under a high power, observe the very numerous **yellow chromoplasts** of more or less distinctly crystalline form

Strip off now a similar patch of tissue from the upper, red portion, mount, and examine as before here the chromoplasts will be seen as before, but masked by the more prominently coloured **red cell-sap**.

THE STAMEN.

All the following preparations should be made from material hardened in alcohol, or fixed with saturated solution of picric acid, and then washed, and hardened in alcohol.

1. Cut transverse sections of a flower bud of *Caltha palustris*, which was just ready to open, taking care that the anthers shall be cut through transversely. Neglecting the other parts, mount the sections of the **anthers** in glycerine, and examine with a low power. Note —

1. The general outline of the section, and compare it with the form of the bilobed anther as above observed

2. The two large cavities or **pollen-sacs** in each lobe.

3. These are surrounded externally by the **wall** of the anther which consists of at least three layers of cells

4. The **septa**, which divide the two **pollen-sacs** or **microsporangia** of each lobe from one another ; the anther has thus originally four pollen-sacs these may be found still distinct in almost mature anthers, though as they approach maturity the septa are partially broken down, and the cavities of the pollen-sacs are thus thrown together this usually happens before the dehiscence of the anther.

5. A single small **vascular bundle** lying symmetrically between the cavities in the central part or **connective** of the anther.

6. **Pollen-grains** or **microspores**, mostly to be found lying free in the glycerine.

Put on a high power and make the following observations —

1 The wall of the anther is composed of —

(a) A layer of **epidermis**, with an external **cuticle**: within this is —

(b) A layer of cells with a **fibrous thickening** of the cell walls

(c) Immediately within (b) will be seen a narrow highly refractive band, consisting of the remnants of two transitory layers of cells, the inner of which was the **tapetum**: this is almost completely disorganized, the outer of the two layers, which abuts on the fibrous layer, is less completely disorganized, and may be seen as an almost continuous layer of thin-walled cells, even in almost mature anthers. Note also that the wall of the anther is thinnest, and its construction most simple at the part most remote from the connective, *i.e.* where the septum of each lobe meets the wall of the anther, while nearer to the connective it becomes thicker

2 At the point where the septum meets the wall of the anther the cells are smaller, and of rounded form, owing to the presence of intercellular spaces between them, and the inner layer is not spirally thickened — this is the **line of dehiscence** of the anther, and the lax character of the tissue at this point helps to bring about the rupture

3 The **pollen-grains** or **microspores**, which are almost spherical, with smooth walls and granular protoplasmic contents, in which may be made out, with difficulty, **two nuclei**.

CARPEL AND OVULES.

1. The following preparations must be made from material hardened in absolute alcohol, or methylated spirit. From an open bud of *Caltha palustris* which has been thus treated, strip off the outer organs, and cut a large number of transverse sections of the **carpels**: by so doing the ovules will be traversed in a longitudinal direction. Treat the sections with one-half pure glycerine, one-half alcohol, in a watch-glass, and let the alcohol evaporate gradually — pick out those sections which appear to have fairly traversed one or more of the ovules, and mount them in pure glycerine.

Examine first with a low power, and observe—

1. The **carpel**, having a structure not unlike that of an ordinary leaf, and consisting of an upper and lower epidermis with some four layers of mesophyll between them. Note the **suture** or junction of the two margins of the carpel, which thus encloses a central cavity.

2. The **ovules** (**macrosporangia**) seated in this cavity, and attached near the margins of the carpel. It has already been noted that there are two rather irregular rows of ovules in each carpel, therefore at most only **two** ovules appear in each section.

The form of the ovule is **anatropous**: it consists of the following parts—

a. The **funiculus**, or stalk, which adheres through the greater part of its course (as the **raphe**) to the body of the inverted ovule. A procambium bundle, connected with a bundle at the margin of the carpel, traverses it longitudinally.

The body of the inverted ovule consists of—

b. Two **integuments**, each several layers of cells in thickness, the outer being united with the funiculus. The integuments cover the body of the ovule completely, excepting a narrow channel (**micropyle**) near its apex. Within the integuments lies—

c. The **nucellus**, an oval mass of cellular tissue in which is embedded—

d. The **embryo-sac** (**macrospore**), a large oval cell, situated centrally a short distance below the apex of the nucellus.

Examine the embryo-sac with a high power, and observe—

i. The granular, vacuolated protoplasm which fills it. Embedded in this are to be found—

ii. A large central **nucleus**, with highly refractive **nucleolus**.

iii. At the micropylar end of the embryo-sac, **three cells**, with clearly defined nuclei. Two of these (the **synergids**) fill the apex of the sac, the third (the **ovum**) being placed laterally, a little below the apex.

iv. At the posterior end of the sac are three cells (the **antipodal cells**), also with clearly defined nuclei. Divisions of these cells occasionally occur, so that their number may be found to be greater than three.

Note the **tapetum**, consisting of cells more or less disorganized which partially or completely surround the embryo-sac.

POLLEN AND POLLEN-TUBES.

I Take some fresh pollen of *Scilla nutans* treat it for an hour or so with solution of methyl green in weak acetic acid wash in weak acetic acid, and mount in the same, or in weak glycerine. Examine with a high power, and observe—

1 The smooth **cell wall**, covering the oval pollen-grain

2 The **granular protoplasm**, which embeds **two nuclei**, which are stained green.

Examine these carefully. One occupies a lateral position, and is of curved and elongated form: it is surrounded by a definite protoplasmic body of oval outline, and is usually deeply stained: this is the **generative cell with its nucleus**. The rest of the grain is occupied by the **vegetative cell and nucleus**: the latter is of round form, and is not so readily stained as the generative: it occupies a more central position, and is not so easily seen.

Pollen of other plants, both Monocotyledons and Dicotyledons, should be compared: the external form and markings vary greatly, but the internal structure remains remarkably uniform, as regards the number and relative position of the parts.

II. In order to observe the germination of the pollen-grains, and formation of the pollen-tubes, cultures may be made in a watch-glass, or, if it is desired to follow the process in single individual grains, use may be made of the moist chamber described in Appendix A.

Mount some pollen-grains of *Scilla* in one hanging drop of a weak solution of cane-sugar in water (about 5 to 10 per cent). Examine them with a high power, and note their form, and the external configuration of their walls.

Keep them at an ordinary temperature in the dark, examine them at intervals of 12 to 18 hours: many will be found to put out **pollen-tubes**, filled with granular protoplasm, in which, after suitable staining, two **nuclei** may be detected, which

stain more deeply these result from the division of the nucleus of the generative cell, and each is surrounded by its own cytoplasm there are thus **two sexual cells**. Behind these may be seen the less clearly stained vegetative nucleus.

FERTILIZATION.

I. Remove from flowers of *Stellaria media*, which have just faded, the three styles moisten them with alcohol, and mount quickly in water note the cylindrical colourless **styles**, curved at their upper ends the **stigmatic surface** with its numerous papillose hairs is found on the convex side of the curved part of the style. Note especially the numerous yellow **pollen-grains** adherent to the stigmatic surface, while it may often be seen that a **pollen-tube** will proceed from the pollen grain, and enter the tissue of the style.

Similar observations may be made upon any plants which have a roughened, papillose stigma.

II. The style and stigma of flowers of *Rhododendron ponticum* from which the corolla has already fallen off will also be found to be good material for showing pollen-tubes. Cut transverse sections of the style in the fresh state, mount in dilute glycerine and observe—

1. The tissue of the style with small vascular bundles dotted in it.

2. The **star-shaped central cavity**, filled with mucilage, embedded in which may be seen—

3. The small **pollen-tubes** cut transversely, and embedded in a mass of transparent mucilage

III. Cut longitudinal sections of the same, including the stigma, and mount as before. observe—

1. The irregular **stigmatic surface**.

2. The numerous **pollen-grains** (associated in groups of four attached to the stigma, and often putting out pollen-tubes which penetrate the tissue of the style.

3. The **pollen-tubes**, often to be seen as a dense sheaf, pursuing their course down the cavity of the style: note their thin walls, and the presence of highly refractive **plasmogones** which

stop their cavities look for endings of the tubes, in which the protoplasm will be denser, and one or two nuclei may be observed there.

IV Pick out gently a number of ovules from an ovary of a flower of *Datura Stramonium*, or of *Digitalis purpurea*, which has just faded, and mount in dilute glycerine Observe -

1 The **campylotropous ovules**, with curved body.

2 **Pollen-tubes**, which are often to be found with the end applied closely to the micropyle

Similar observations may also be made on *Stellaria media*, and many other plants, in which the ovules are numerous and small.

V In order to recognise the influences which affect the growth of pollen-tubes, cultures in a hanging drop may be used Arrange a **moist chamber**, as directed in Appendix A Into a drop of 10 per cent sugar solution, introduce a short piece of the style of *Scilla nutans*, including the stigma also a few quite fresh pollen-grains Mount and examine at intervals of half an hour Very soon the pollen-tubes will begin to be formed, the time depending upon the temperature, and other conditions at first the pollen-tubes have no definite direction but in two or three hours many of them will curve round, so as to approach the piece of the style, and then ends will penetrate its tissue at either end The style has, in fact, a positive **chemiotactic influence** upon the growing tubes.

Note also that the majority of the tubes will turn away from the margin of the cover-slip they are **negatively aerotropic**.

Experiments may also be arranged to show that they are susceptible to light-stimulus.

RESULTS OF FERTILIZATION

A. DEVELOPMENT OF THE EMBRYO.

i. *Dicotyledon*

Take a fresh ovary of *Capsella Bursa-pastoris*, which has attained about a third the ultimate size of the mature fruit open it with a needle, and pick out upon a slide a number of the ovules.

Material kept in spirit will not do well for this work, and the specimens should be in good fresh condition, not withered.

Treat the ovules at once with dilute potash, they will become more transparent as the reagent permeates them. Examine them with a low power and observe—

1 The form of the ovule (**campylotropous**, *i.e.* with a curvature of the body of the ovule)

2 The **funiculus**, or stalk.

3. The **integuments**.

4. The **micropyle**, not very easily seen a **pollen-tube** may often be observed entering the micropyle

5. A large central cavity (the **embryo-sac**), which is curved like the whole ovule In this may be seen, more or less distinctly—

6 The **embryo**

To study the structure of the embryo, either longitudinal sections of the ovule must be cut, and the embryo be thus laid bare, or the embryo must be removed from the ovule The former is the more accurate method, though the latter is much the easier the latter will therefore be adopted.

Press gently with a needle upon the cover-slip of the above preparation, so as to burst the ovules. the embryo will escape in some cases without injury but this will only be the case when fresh material has been used ; after hardening in alcohol the embryos will not readily leave the ovule. Neutralize the potash with dilute acetic acid The structure of the embryos, which now lie freely suspended in the fluid, may be easily studied, and as the acetic acid causes the protoplasm of each cell to contract, the cellular construction of the embryo can be clearly seen.

Apply the same method for the preparation of embryos, from ovaries of various ages, both younger and older than that first taken. A series of preparations may thus be obtained illustrating various stages of development of the **embryo**, such as are figured in ordinary text-books.

Note more especially the following successive stages of development.—

1. The **suspensor**, consisting of one or more cells and terminated by a single **embryonic cell**.

2. The embryonic cell divided into **octants** arranged in two tiers. the suspensor is elongated and the cells divided so as to form a series, of which the basal cell (that nearest the micropyle) is usually enlarged greatly, so as to exceed the embryo in size, and beginners are apt to mistake it for the embryo: the terminal cell next the embryo (the **hypophysis**) encroaches between the four lower octants of the embryo.

3. The octants so divided as to form three layers of cells, which have been distinguished as (*a*) the external **dermatogen**, (*b*) the **periblem**, (*c*) the central **plerome**

4. The two **cotyledons** formed by lateral outgrowth from the upper tier of octants, the apex of the **radicle** derived from the hypophysis, the hypocotyledonary stem from the lower tier of octants

5. Other parts as before. The **apical bud** or **plumule** formed between the cotyledons.

ii. *Monocotyledon.*

Treat ovules of *Alisma Plantago* in the same way, and observe the following stages of development —

1. **Suspensor** and **embryo** consist of a single short series of cells, produced by transverse divisions

2. The terminal cell divides longitudinally into four (first tier).

3. The second, third, and fourth cells from the end also divide successively (second, third, and fourth tiers).

4. The cells of the body of the embryo divide (as in *Capsella*) so as to form three layers: (*a*) external **dermatogen**, (*b*) **periblem**, (*c*) central **plerome**.

5. A lateral depression of the surface, at the level of the second tier. At the basal lip of this the **apical cone** of the plumule is formed, and is thus lateral.

The single **cotyledon** is formed from the first tier, and is thus terminal.

The **radicle** from the third tier.

The **apex** of the root from the fourth tier.

Compare these results with those obtained in *Capsella*.

B. DEVELOPMENT OF THE ENDOSPERM.

I. This may be traced in the embryo-sac of *Caltha palustris*, in material which has been fixed and preserved in absolute alcohol or strong methylated spirit it is an advantage to collect the material on a hot day, and place it in alcohol without delay ; by this means many nuclei may be fixed in various stages of division.

II. Cut transverse sections of the carpels of a flower of *Caltha palustris* which is full blown, or even beginning to fade, and also sections of successively older specimens up to the almost mature fruit. mount them in glycerine, and compare them. they may illustrate the changes which appear in the embryo-sac subsequently to fertilization, viz --

1. The penetration of the micropyle and apex of the nucellus by the pollen-tube.

2. The first stages of development of the embryo, which in this case remains relatively small, the seed being an albuminous one ; the embryo will thus be seen *in situ*.

3 The division of the central nucleus of the embryo-sac into two, subsequently into four, eight, &c.

4 The disposition of the nuclei, as they increase in number, as a dense series embedded in the protoplasmic film at the periphery of the embryo-sac.

5 The formation of cell-walls between these nuclei, so that the embryo-sac is lined internally by a single layer of cells of the endosperm

6. The ingrowth of these cells, and their subsequent division so as to fill the cavity of the embryo-sac with endosperm, which embeds the embryo

7. The great increase in size of the embryo-sac, and of the whole ovule

8. Note also the changes in the integuments, and the disappearance of the nucellus as the ovules become mature.

On looking over a number of such sections, numerous cases of **division of nuclei**, illustrating various stages of the process, may be found. These points may be very well studied in the embryo-sacs of *Fritillaria imperialis*, &c.

III. The continuity of protoplasm through cell-walls has been shown in the sieve-tubes (p. 63). Similar observations of fine threads of protoplasm traversing the cell-walls may be made in the endosperm of various seeds, and these are merely prominent examples of a widespread phenomenon.

Cut ~~thin~~ sections with a dry razor from the endosperm of the dry seed of *Strychnos Nux-vomica* : first mount a section in glycerine, and observe, under a high power, the dense protoplasmic body of each cell surrounded by a thick cellulose wall.

Mount other sections in *tincture of iodine not diluted with water* : then place a small drop of water at the edge of the cover-slip, drawing it under by means of blotting-paper : observe the edges of the section where the effect of the dilution will first appear, and as the cell-walls swell, it will be seen that the substance is traversed transversely by fine threads of protoplasm, which are stained by the reagent.

Another method has been found to succeed well in demonstrating continuity through the cell-walls of the endosperm of various Palms, in which the endosperm has thick pitted walls consisting of reserve cellulose : it is, to immerse the fresh sections in sulphuric acid in which a small quantity of powdered Hoffmann's blue has been dissolved : when the sections are sufficiently acted upon, wash them with water and mount in glycerine. The protoplasm will be stained a deep blue, and the swollen cell-wall is not stained : examine the swollen pit-membranes, and if the treatment has been successful they may be seen to be traversed by fine curved strands of stained protoplasm.

RESERVE AND TRANSITORY MATERIALS

A—IN THE VEGETATIVE REGION

I Examine a young Potato plant (*Solanum tuberosum*) dug up in early summer. note the green shoots above ground, with the leaves, and leafy axillary buds. Follow the axis downwards, and note in the axils of such lower leaves as may be underground that the **axillary buds** take an elongated, cylindrical form, with more or less swollen ends. these are the young **tubers**. Examine one, and note the distended fleshy axis, and small-brown **scale-leaves**: in the axils of these appear **axillary buds**, the **eyes**.

Cut a young Potato transversely, and note with a lens, or low power, the fleshy, parenchymatous tissue, traversed by small vascular bundles, which are disposed in a ring, as is typical of Dicotyledon stems; but here the stem is greatly distended.

The storage materials are -

(a) **Starch**: for its recognition and characters see p. 31, III.

(b) **Proteid-crystalloids**: these are of cubical form, and occur chiefly towards the periphery. Cut tangential sections from material hardened in alcohol, or in picric acid and alcohol, and mount in pure glycerine and iodine the cubical crystalloids will be distinguished by their yellowish brown staining.

II Examine the swollen stem (corm) of the common *Crocus*, as sold in shops in autumn. It is disc-shaped, and covered externally by dry membranous scales. at the base is a circular scar, around which the roots may already be seen. At the apex a central bud, surrounded it may be by others, which are plainly axillary. Cut the stock transversely, and note the vascular bundles scattered through the massive conjunctive tissue, as in ordinary Monocotyledons.

Cut thin transverse sections, and stain with iodine: the numerous starch-grains, which crowd the conjunctive cells, stain blue.

III. **Leukoplasts, or Starch-forming Corpuscles.**—These are to be found in colourless tissues, in which a storage of starch is taking place; *e.g.* a young Potato, the rhizome of *Iris*, or *Canna*, &c

Cut up very young Potatoes into small pieces, and treat with picric acid: wash out carefully with dilute alcohol, and harden. Cut sections parallel to the surface, and not far below it, since the leukoplasts are best seen in the more superficial cells. Treat the sections with very slightly diluted tincture of iodine, and mount in pure glycerine examine under a high power, and observe —

1 The cells of the usual parenchymatous type, with protoplasm and nucleus.

2 Numerous spherical bodies, usually aggregated round the nucleus, and stained yellow or brown these are the young **leukoplasts**, which have not yet formed starch. Older leukoplasts may be found attached to starch-grains, which they have formed internally the starch-grains, growing larger, and staining blue, are then the more prominent objects.

The Potato is not such good material for demonstrating the leukoplasts as the tuberous swollen stems of an Orchid, *Phajus*. Sections may be treated as above, and the leukoplasts will be seen as rod-shaped bodies of considerable size.

IV Compare with the Potato the tuberous underground stem of the Jerusalem Artichoke (*Helianthus tuberosus*) it is of irregularly pear-like shape, the narrower end being the connection with the parent plant, from which it arises as an axillary branch. Observe the broad, filmy, brown scale leaves, in the axils of which again arise conical axillary buds, bearing further scale leaves. Roots are attached to the stem at irregular intervals.

Cut transverse sections of the thin stalk: treat some with chlor-zinc-iodine, others with glycerine examine with a low power, and observe that the general structure is like that of the Sunflower stem (see p. 49), the Artichoke being another species of the genus *Helianthus*. The most obvious differences are, the very small quantity of sclerenchyma present, and the prevalence of transparent parenchymatous tissue.

Cut transverse sections through the swollen tuber, and compare them with those of the stalk. Note that a slight hypodermal cork may be present: the sclerenchyma is absent from the pencycle. There is a bulky phloem as before, but the xylem is dissociated into smaller groups, owing to the predominance of xylem-parenchyma and medullary rays, which are here unligified; the mass of parenchyma thus formed is continuous with the bulky pith. The parenchymatous cells retain their protoplasm and nuclei, but contain **no starch**.

Cut sections from the fresh, transparent tuber: place them in a watch-glass in alcohol, and note that the tissue becomes opaque: examine a section under the microscope, and the cells will be found filled with a granular precipitate. Wash out with water, and they become again transparent. The precipitate consists of **inulin**, which is insoluble in alcohol, though soluble in water.

Cut sections from material which has been kept in alcohol to precipitate the inulin, and mount in glycerine. Observe the tissues as before. but throughout the section are numerous **sphere-crystals of inulin**, irregularly disposed, and varying in size. Note the radial construction of these crystals, and that they often include cell-walls, or even whole groups of cells.

Treat the section with water: the crystals dissolve slowly, disintegrating along radial lines.

Treat with potash solution: the crystals dissolve without colouration, but more quickly than in water.

Treat with iodine solution: the crystals are not coloured.

V. Dig up a plant of the common garden *Dahlia* (*D. variabilis*), and note that the roots are swollen into long pear-shaped tubers, narrowing off at the apex and base into regions of normal thickness.

Cut one of the tubers transversely, and note with a lens the soft sappy tissue, traversed by vascular strands.

Cut transverse sections of the thin portion of the root, and observe the root-structure, with polyarch xylem, and secondary thickening. There is a large central pith.

Cut transverse sections of the swollen tuberous root, which has been preserved in alcohol, and note with a low power the

numerous **sphere-crystals of inulin**, as before they will show the same reactions.

The bulk of the section is chiefly due to increase of parenchyma in the secondary xylem : small groups of secondary tracheae may be observed scattered throughout the parenchyma. Near the central pith the primary strands may still be recognized. Note also the well-developed protective coat of cork at the periphery of the section.

VI. Cut the swollen tapering root of a common Carrot (*Daucus Carota*) transversely note the soft, succulent mass, divided by a definite cambium-ring into a central yellow xylem-tract, and a peripheral red tract of secondary phloem. The increase in bulk is due to parenchymatous distension of both the xylem and phloem, the latter being very bulky.

Cut transverse sections from the fresh Carrot stain with iodine solution, and note that starch is present in considerable quantities, especially in the secondary phloem

Cut some small slices of Carrot, boil them in water in a test-tube, add a few drops of Fehling's solution (see Appendix A), and boil gently a brick-red precipitate will be formed, showing the presence of **Grape-sugar**.

VII. Cut a Turnip (*Brassica Napus*) transversely, and note that the enormously distended axis is mainly composed of parenchyma, dotted with strands of xylem. a cambium will be seen with a lens, near the periphery, and the chief bulk is thus due to parenchymatous distension of the xylem, rather than the phloem.

Stain a transverse section with iodine solution. **starch** may be found throughout the parenchymatous tracts.

If tested with Fehling's solution, **Grape-sugar** will be detected.

VIII. Cut the swollen conical root of the garden Beet (*Beta vulgaris*) transversely, and note the successive rings of xylem and phloem, which, with a very large proportion of succulent storage-parenchyma, form the massive root.

(1) Cut transverse sections : mount them in water, and note under a low power the transparent tissue, and coloured cell-sap. Treat such a section for a few minutes with alcohol in a watch-glass : on re-examination under the microscope, crystals of

small size will be seen in the cells. On irrigation with water they will be re-dissolved. These are crystals of **Cane-sugar**.

(2) Boil some small pieces of Beet-root in a small bulk of water. pour off the coloured extract, add to it a little of Fehling's solution, and boil. no precipitate will be formed.

(3) This point may be further verified by testing sections as directed above for the Carrot. no precipitate will be formed, either in the cells, or in the surrounding fluid. These reactions will serve to distinguish Cane-sugar from Grape-sugar.

Similarly, the presence of Cane-sugar may be demonstrated in the Sugar-Cane itself.

IX. Examine a common Onion (*Allium Cepa*) it is a tunicated bulb, covered externally by dry, papery, and completely sheathing **leaf-bases**, which are, together with the fleshy leaf-bases within, inserted upon a short **axis**. the latter may be recognized at the base of the bulb, and from it numerous roots project. Cut the bulb vertically in two, and observe that it is composed of—

(1) The axis, which is bluntly conical, and of small size. its upper surface is entirely occupied by the insertions of —

(2) The bases of the leaves. of these the inner are fleshy, for purposes of storage. the outer are dry, membranous, and protective. Note at the top of the bulb the dried remains of the upper assimilating portions of the leaves.

Cut a section through one of the succulent leaf-bases, and note the transparent watery character of the tissues. Stain with iodine solution. starch will not be found.

Treat as above with Fehling's solution, and the precipitate on boiling will demonstrate the presence of **Grape-sugar**.

B.—IN FRUITS AND SEEDS

I. Examine a ripe Grape, the fruit of *Vitis vinifera*. it is a superior berry. note the succulent pericarp, with superficial skin, and the seeds, with hard testa, embedded in the pulp.

1. Cut a transverse section of the ripe pericarp, of such thickness that some cells at least shall be uninjured. mount in

water, and observe under a low power the transparent parenchymatous pulp, consisting of cells with thin walls, very sparing contents, and large central vacuole

Treat for a few minutes with a relatively large bulk of alcohol in a watch-glass on re-examining, numerous crystals will now be seen in the cells they are larger than those of the Beet.

Irrigate thoroughly with water the crystals may be seen to be re-dissolved. they consist of **Grape-sugar**, which is in solution in the cell-sap of the living cell

2. Squeeze out the juice of some Grapes into a test-tube add a little of Fehling's solution (see Appendix A), and boil a bulky reddish precipitate is formed owing to reduction of the copper.

3. Soak a fairly thin section of a Grape in Fehling's solution wash quickly with water, mount in water, and boil gently over a spirit-lamp a precipitate like the above (2) is formed note under the microscope that the dark-looking granules of the precipitate (cuprous oxide) are to be found actually within the cells of the tissue, thus indicating that the sugar was there

II. **Cellulose** occurs as a reserve in the endosperm of the Date, and other Palms for the structure of the Date "stone," see p. 42 (d) for the chemical reactions of cellulose, see p. 26, A.

Sections should be cut and the reactions noted. Thickened cell-walls will also be found in sections of the cotyledons of *Lupinus*.

III. Examine the Common Almond of the shops It is the seed of *Amygdalus communis*, the fruit of which is a deliscent drupe: the shell, which is often present in shop Almonds, is the endocarp. The brown skin of the Almond is the testa, which immediately invests the large embryo, the seed being exalbuminous Note the two cotyledons, plumule and radicle.

Cut thin sections of the cotyledon of the Almond mount in water, and note the bright-looking oil-drops, both in and about the section, and dispersed also in the water.

1. Irrigate with alcohol the drops are not dissolved

2. Treat a section with a considerable bulk of ether in a

watch-glass : wash with alcohol, and mount in alcohol, or in glycerine : on examination the oil will be found to have been dissolved by the ether.

3. Stain a thin section with tincture of alkanet (see Appendix A), the oil-globules stain pink.

4. Treat a section with 1 per cent. solution of osmic acid : the oil-drops will stain slowly, taking a dusky or black hue.

5. Treat a section with potash solution, and warm : the oil will be partially and slowly saponified, and dissolved. This effect of potash is best seen in specimens where the oil is present only in small quantities as isolated globules

Oil occurs frequently as a storage material, replacing, partially or completely, the carbohydrates. As further examples of oily seeds, see *Helianthus*, *Arachis*, *Bertolletia*, &c.

IV. The structure of the seed of *Ricinus* has been described above. see p. 39 (*it*) In the endosperm **aleurone grains** of large size are found.

Cut through the endosperm transversely, and with a razor wetted with olive-oil, or castor-oil, cut thin sections from it. mount them in the same oil. Examine under a high power, and observe—

1. The thin cell-walls of the oval cells.
2. The numerous highly refractive **aleurone grains** in each cell ; each grain is of oval form, and a less highly refractive area is seen at one end : this is the **globoid**.
3. The oily protoplasm in which the grains are embedded, this being so transparent as to be hardly visible.

Cut other sections with the razor wetted with alcohol, soak them well in alcohol in a watch-glass to remove the oil, which is soluble in alcohol, but more readily in ether. This solubility in alcohol is unusual. Compare oil of Almond, above, III. 1. If ether be used, wash it off afterwards with alcohol : mount in pure glycerine : examine the sections under a high power, and observe the aleurone grains as before.

1. Add water gradually, and watch its effect on the grains.
 - a. The outer **amorphous coat** of the grain will swell, and become less highly refractive : thus there will be disclosed—

- b. The **crystalloid**, one, or rarely more, being included in each grain · these do not swell greatly with water, and accordingly they retain their refractive power.
- c. The **globoid** will also be visible.
2. Add dilute potash solution · both the amorphous coats and the crystalloids will swell and dissolve, leaving the globoids.
3. Add strong acetic acid · the globoids will dissolve slowly.

Aleurone grains are a form of storage of **proteid material**: they are usually largest in oily seeds, such as *Ricinus*. The crystalloid and amorphous coat are proteid, the globoid is composed of mineral matter. The grains are frequently smaller and of simpler structure, especially in starchy seeds. Sections should be cut of the Bean, where the starch grains are large, and the aleurone grains very small · of *Arachis*, in which the storage material consists of starch, oil, and aleurone grains · of *Lupinus*, where the cell-walls are thickened, starch is absent, and aleurone grains, of some size, are embedded in the protoplasm.

GYMNOSPERMS

VEGETATIVE ORGANS

EXTERNAL CHARACTERS

TAKE a branch of *Pinus sylvestris*, cut in autumn, including at least four years' growth. The limits of each year's growth may be recognized externally at those points where false whorls of strong lateral axes are developed; and the portion of stem lying between two such whorls may be regarded as roughly representing one year's growth.

1. Consider first the growth of the year in which the branch was cut, *i.e.* the part above the youngest whorl of lateral axes. At its apex is a large **bud**, surrounded by a variable number of smaller **lateral buds**.

From a bud, which has been treated with alcohol to remove the external secretion of the resin, detach some of the brown **scale-leaves**, which cover it externally. Note —

1. The succulent base of these scales.
2. Buds in their axils.

In studying the growth of the current year, bear in mind that it has been derived from a bud which had a similar structure to that which is now seated at its apex. Examine the stem of the current year externally, and note —

1. The thick **main axis**, more or less succulent in appearance. Its surface is marked by longitudinal grooves.
2. The persistent brown tooth-like bases of the scale-leaves of the bud, the upper part of which had fallen in spring: they are best seen at the lower part of the internode.

3 In the axils of these, especially at the upper part of the internodes, are **axillary buds** of two kinds

(a) Buds with limited growth (**foliar spurs**), each bearing in this species two acicular **foliage-leaves**, surrounded at the base with numerous scale-leaves. These dwarf foliage shoots occur in the axils of the scales throughout the greater part of the current year's growth; in older parts they may be found to have fallen off, the foliar spurs separating as a whole from the parent branch.

(b) Buds with unlimited growth, which are seated close to the apex of the shoot of the current year. They are few in number; their structure has already been observed; each may develop into an unlimited axis. The general habit of the tree is defined by these axes of unlimited growth.

It may here be observed that (a) and (b) have a similar origin, both being axillary buds in the axils of the leaves of the main axis of the current year. The apparent difference depends upon the fact that the buds (b) are more strongly developed than (a).

II. Passing to the increments of growth of former years, *i.e.* to the lower and older parts of the branch, in the external appearance and arrangement of parts they resemble that of the current year. The main axis increases in thickness, and is more obviously woody, while the foliar spurs drop off, leaving scars which mark their former position.

THE STEM

It is best to work with material which has been treated for some time with spirit; by this means the resin, which would otherwise clog the razor, is removed.

I. Cut transverse sections of the young elongating shoot of the current year, taken in June—mount some in glycerine, others in chloro-zinc-iodine—the sections have a wavy outline, the indentations corresponding to the grooves which may be observed externally. Starting from the periphery of the section, note the following tissues.—

1. **Epidermis**, a single layer of cells, following the wavy outline of the section; the walls, especially the outer, are much thickened. externally there is a well-marked **cuticle**.

2. **Cortical tissue**, consisting mostly of cells having rather thick cellulose walls (blue with chlor-zinc-iodine), and protoplasmic contents with chlorophyll. Other cells have thicker and firmer walls, staining yellow with chlor-zinc-iodine. Many cells have recently divided. this is necessary to keep pace with the growth in thickness of the vascular cylinder

Here and there in the cortex large **resin-passages** occur they are essentially enlarged intercellular spaces, the cavity being lined by a layer of delicate, densely protoplasmic cells—the **epithelium**—which secrete the resin

Near the periphery of the cortex may be found a layer of **cork** and a **cork-cambium** (compare stem of Elm, p. 79), derived from cells of the cortex by their division by tangential walls. The mature cells of the cork have no cell-contents their walls are coloured yellowish brown with chlor-zinc-iodine.

3. The **stele**, similar in general structure to that of a Dicotyledon, in so far as the central **pith** is surrounded by a ring of **vascular tissue**: there is no definite external limit to the stele, owing to the absence of any characterized endodermis. nor can any distinct pericycle be recognized.

In sections from near the apex, the vascular ring is more or less distinctly divided into so many primary vascular **bundles**, the large medullary rays separating them being due to the departure of strands to the scale leaves and foliage spurs. But as in Dicotyledons (see p. 68), secondary thickening sets in so near the apex, that the distinction between these bundles is soon lost, and the vascular tissues form an apparently continuous ring, as is the case in thin sections of the elongating shoot of the current year. In this vascular ring distinguish the external **phloem**, with its bright-looking cellulose walls (blue with chlor-zinc-iodine); the internal **xylem**, the components of which have thick dark-looking lignified walls (yellow with chlor-zinc-iodine), and the misty layer of **cambium** between them.

Observe that the internal limit of the vascular ring is sinuous :

the convexities mark the position of the primary bundles. at the inner limit of these will be found the **protoxylem**.

The **pith** consists of parenchyma, having the same characters as in the cortex. there are no resin-passages.

Put on a high power, and examine the **cambium**. Note—

i. That the cells are arranged with great regularity in **radial rows**.

ii. That their walls are thinner than those of the surrounding tissues, and are composed of cellulose (blue with chlor-zinc-iodine)

iii. That the tangential walls are thinner than the radial.

iv. That the cells have copious protoplasm, in which a nucleus may often be recognized.

These facts point to a repeated division of cells by tangential walls. (Compare Fig 5, A, p 77)

Observe, here and there, radial rows of which the cells are more elongated in a radial direction than the rest. these may be traced outwards towards the cortex, and inwards towards the pith. they are the **medullary rays**. (Compare Fig. 5, A, row 2) Some of them may be traced the whole way to the cortex and to the pith (**primary medullary rays**), others only part of that distance (**secondary medullary rays**)

Follow the radial rows of cambium-cells outwards, and note the gradual transition to the permanent tissues of the **secondary phloem**, the constituents of which are also arranged in radial rows, and have cellulose walls (blue with chlor-zinc-iodine)

Observe the following constituents of the phloem —

i. Elements with cellulose walls, and no very distinct contents: they are radially compressed. these are the **sieve-tubes**, which compose the greater part of the phloem

ii. Here and there the radial rows of sieve-tubes are broken by single large cells of the **phloem-parenchyma**, which resemble in their characters those of the medullary rays

iii. Towards the periphery of the phloem the earlier formed sieve-tubes are seen in various stages of disorganization.

Note on passing to the periphery of the phloem, an increasing irregularity of form of the tissues, due to distortion, caused by pressure from without by the cortical tissue upon

the vascular system, as it increases in bulk by secondary thickening

Follow the radial rows of cambium-cells inwards, *i.e.* towards the centre of the stem. Note the transition from thin-walled cambium to the thick-walled tissue of the **xylem**. If the stem was cut in winter the transition will appear sudden, if cut in summer it will appear gradual

Observe that the xylem-ring is cut by the medullary rays into wedge-shaped areas. The chief tissue-elements filling these areas are the **tracheides**, which present the following characters —

- i. They have approximately the same shape as the cells of the cambium from which they are derived.
- ii. Their walls are thick and lignified (yellow with chlor-zinc-iodine), and are differentiated into layers, distinguished optically and by staining.
- iii. They have no cell-contents.
- iv. On their radial walls (and rarely on the tangential walls) are found the **bordered pits**, which are best seen in the xylem formed at the early part of the year

These appear, when seen in transverse section under a low power, as biconvex enlargements of the wall, which look darker than the rest of the wall, under a high power it is seen that there is a biconvex-lens-shaped **pit-cavity**, over-arched on either side by a meniscus-like outgrowth of the wall, which, however, does not form a complete cover, for a pore on either side leads into the pit-cavity. The cavity of the pit is traversed by the thin **pit-membrane**: there is a slight swelling at the middle of the pit-membrane called the **torus**. The pit-membrane sometimes runs straight across the pit, but often it is curved to one side. The wall is thus not perforated, but the pit consists of a thin persistent region of the wall (the pit-membrane), partially over-arched by a thickening growth.

Observe near the centre, and bordering on the pith, the **protoxylem**.

Note the occurrence of **resin-passages** in the secondary xylem, lined as before by thin-walled epithelium, which may be regarded as a form of **xylem-parenchyma**.

- II. Cut transverse sections of a **three-year-old stem** so as to

include the whole width of the vascular ring : it is not necessary however to have a complete transverse section of the whole stem. Mount in glycerine. Comparing this with what has already been observed in the stem of the current year, note the following differences —

1. The cortical tissue bears evident traces of tangential extension. This is necessary to keep pace with the increase in bulk of the vascular system.

2. The phloem is thicker, and the constituents of the outer part of it are much distorted and displaced

3. The xylem has increased in thickness more than any other tissue, so that it is now the chief constituent of the stem. It may be distinguished as being composed of three bands (**annual rings**), in each of which the more central tracheides have large cavity and thinner walls (wood developed in spring) passing outwards through the annual ring a gradual reduction of the cavity may be seen, and increase in thickness of the walls till a certain limit is reached (autumn wood).

Outside the latter is a sudden transition to the spring wood at this point is the limit of each year's growth

III. Cut radial longitudinal sections of a three-year-old stem : mount some in glycerine, others in chlor-zinc-iodine. The section should be accurately radial and longitudinal, otherwise the difficulty of study of the tissues is greatly increased

Beginning at the centre of the stem and passing outwards, observe successively—

1. The **pith**, consisting of two sorts of elements, both of which are of parenchymatous form.

- a. Cells with pitted cellulose walls, and having protoplasm and nucleus.

- b. Elements of similar form with pitted lignified walls, and no cell-contents.

2. The **xylem**, consisting of—

- a. **Tracheides** with lignified walls, and no cell-contents. Starting from those nearest the pith, and passing outwards, the following forms may be observed :

- i. Tracheides with narrow cavity, and more or less regular annular or spiral marking—the **protoxylem**.

- ii. Elements wider than these, and with bordered pits scattered between the spirals
- iii. Normal **tracheides**, with bordered pits only - these form by far the greater bulk of the secondary xylem, and must be carefully studied. Their form is **prosenchymatous**. The greater part of the cell-walls is of uniform thickness. On these portions of the wall observe with the high power two intersecting systems of **lines of striation**. The **bordered pits** are found in single longitudinal rows: each pit has the appearance of two concentric rings, of which the smaller is more strongly marked, and corresponds to the opening of the cavity of the pit into the cell-cavity, the larger represents the limit of area of the pit. It must be remembered that we are now observing the radial walls in surface view. A careful comparison should be made of the bordered pit as seen here in surface view with its appearance when seen cut through, which is the view presented in the transverse sections above described

Note the **annual rings**, recognized here as in the transverse sections, the autumnal wood being distinguished by the smaller size of the cavity and greater thickness of the walls of the tracheides.

b. Here and there the continuity of the mass of tracheides is broken by a longitudinal **resin-passage**, surrounded by parenchymatous cells, which have cellulose walls and retain their cell-contents

c. The whole mass of xylem is traversed radially by plates of parenchyma (**medullary rays**). Note that they extend only a short way longitudinally, but a long way radially. also that they are composed of cells arranged like bricks in a wall, among which may be distinguished—

- i. Cells with cellulose walls, and protoplasmic contents, with starch: the pits in the walls of the tracheides which abut on these are unusually wide, and only slightly bordered.
- ii. Elements of very irregular form, having no protoplasm, and with irregularly thickened, lignified walls, marked

with bordered pits. Both tissue-forms are commonly found in the same ray, the **starch-bearing cells** (i) being towards the middle, and the **tracheidal cells** (ii) being at either margin. Note that between the starch-bearing cells clearly defined lines may be seen running radially. these are **intercellular spaces**.

3. The **cambium-layer**, consisting of elongated thin-walled cells, the ends of which are difficult to observe. They have copious protoplasm, and an elongated nucleus. (Compare Fig. 5, p. 77.)

Note that the medullary rays are continuous through the cambium, and observe the differentiation from the uniform cambium of the ray to the cell-forms (i) and (ii).

4. The **phloem** tissues, which are best studied in sections which have been treated for some hours with chlor-zinc-iodine, consist of—

a. **Sieve-tubes**, elongated structures with cellulose walls, those which are radial being marked by numerous circular **sieve-plates**, here seen in surface view. these sometimes stain a sherry brown with chlor-zinc-iodine. The ends of the tubes are difficult to observe. their protoplasmic contents are transparent and sparing. The sieve-plate is not a simple one, but is subdivided into a number of very minutely perforated areas.

b. **Phloem-parenchyma**, cells arranged in longitudinal rows, with cellulose walls, and copious protoplasm. Occasional elements (prosenchymatous or parenchymatous) are found with brown cell-contents, in which **crystals** are embedded. these are found towards the periphery of the phloem.

c. **Medullary rays**, radially traversing the phloem, as in the xylem (see par. c, p. 160). the cells in the middle of the ray resemble (i) in the xylem, but the tracheidal cells at either margin are here replaced by **albuminous cells**, which are elongated vertically, with cellulose walls, and dense protoplasmic contents: they replace the companion cells, which are not present in the phloem of Gymnosperms.

5. Externally to the phloem is the **cortical parenchyma**, which requires no further notice here. Outside this is cork. At the periphery of the section there may still be the **epidermis**.

IV. Cut tangential sections of a three- or four-years-old branch, and bear in mind that as a rule the central part of the sections is the most accurately tangential, *i.e.* that the plane of section is there most accurately perpendicular to the radius of the stem (See p. 6.) Sections should be cut at different depths in the tissues, so that the middle of the plane of section shall traverse (a) the peripheral part of the xylem, (b) the cambium, and (c) the inner part of the secondary phloem. Mount as before.

a. In sections which pass through the peripheral part of the **xylem** observe—

i. The **tracheides**, of prosenchymatous form. No bordered pits (or very few) are seen in surface view, but they may be seen in large numbers in the radial walls (here cut longitudinally) presenting a similar appearance to that seen in transverse sections.

ii. **Medullary rays**, which resemble a section of a biconvex lens. Note that each ray extends only a short distance in a longitudinal direction: in some cases rays consist of only a single radial series of cells, of which only one lenticular cell appears in this section. In those rays which consist of several rows of cells, note the starch-containing cells in the middle of the ray, with pits of large area in their lateral walls: and the tracheidal cells at either margin, with smaller bordered pits. Observe also the small triangular **intercellular spaces** which intervene between the cells: these are only seen in thin sections. Occasionally a **resin-passage** is included in a ray.

iii. **Longitudinal resin-passages.**

b. In sections passing through the **cambium** will be seen—

i. The **cambium-cells**, resembling the tracheides in form (prosenchymatous): the cell-walls are thin, and the protoplasm granular, with elongated nucleus.

ii. The **cambium of medullary rays** is similar in shape to the cells of the rays: it is thin-walled, with granular protoplasm and nucleus. (Compare Fig. 5, C, p. 77.)

If these sections be treated with dilute potash, and mounted in glycerine, their structure may be more easily made out.

c. In sections passing through the **phloem** will be seen—

i. The **medullary rays** in outline as before, but their form is

more convex : all the tissues between the medullary rays are derived from cambium-cells of the form above observed. These are—

ii. **Sieve-tubes**, which retain the form of the cambium-cells, the cellulose walls seen in surface view, *i.e.* the tangential walls, are smooth : those cut longitudinally, *i.e.* the radial, appear of wavy outline, the thinner regions being the sieve-plates ; in which the secondary areas may sometimes be observed. The structure is well seen after treatment with chlor-zinc-iodine for twenty-four hours.

iii. **Phloem-parenchyma**, derived from cambium-cells by their division by transverse walls.

iv. Some few cells, especially towards the periphery, containing **crystals** which give the reactions of calcium oxalate.

THE LEAF.

Examine the foliar spur of *Pinus sylvestris* as a whole. It consists of a very short axis, at the base of which are borne membranous sheathing scales, and at the apex two long needle-shaped foliage-leaves : in other species of *Pinus* the number may be larger. Note that the inner or morphologically upper surface of the leaf is flat, while the outer or lower surface is rounded, and the whole leaf is traversed from end to end by two sharp marginal edges which are slightly rough to the touch.

Cut transverse sections of a foliage-leaf taken from a stem of the current year. It may be found convenient to embed in paraffin, or to hold the leaf between pieces of pith, or carrot. Mount some in glycerine, others in chlor-zinc-iodine, and examine with a low power. Note the semilunar form of the section : the flat side is the upper, the convex side the lower. Starting from the periphery observe successively the following tissues.—

1. A single layer of **epidermal cells** with very thick walls. enlarged cells are to be found at the two corners, and since these cells project slightly they cause the roughness above noted.

2. A narrow band of thick-walled **hypoderma**.

3. A broad band of chlorophyll-containing **mesophyll**, with resin-passages.

4. An **endodermis**, consisting of oval cells, surrounding a central vascular strand.

5. A broad band of **conjunctive tissue**, without chlorophyll, which surrounds—

6. Two central **vascular bundles**.

Study these several tissues under a high power.

i. The **epidermal cells** have their thick walls differentiated into three layers. These may be recognized without staining, or better after treatment with chlor-zinc-iodine, as—

i. A thin external **cuticle**, not very deeply stained: it extends as wedge-like processes between the cells.

ii. The **cuticularized layers**, forming a thick band, which stains a deep brown. Immediately surrounding the cell-cavity is—

iii. A broad pitted band, not deeply stained

Here and there depressions of the external surface may be observed. These indicate the position of the **stomata**. Observe the two **guard-cells**, which are seated some distance below the surface of the leaf: an intercellular space is to be seen immediately below each stoma.

2. The **hypoderma** (sclerenchymatous), varies in thickness from a single layer of cells to several layers. It is thickest at the corners of the section: the cells are thick-walled, and lignified. Note that it is absent below the stomata.

3. The **mesophyll** consists of thin-walled, chlorophyll-containing parenchyma: the cellulose walls (blue with chlor-zinc-iodine) show a peculiar in-folding—**arm-parenchyma**. **Resin-passages** occur in it: their cavity is lined with thin-walled epithelium, which is immediately surrounded by a layer of thick-walled sclerenchyma.

4. The **endodermis** has its radial walls stained brown with chlor-zinc-iodine.

5. The **conjunctive tissue** immediately within this consists of three sorts of elements—

i. **Parenchymatous cells**, with thin cellulose walls (blue with chlor-zinc-iodine), and living contents, with starch

- ii. Elements having lignified walls, with bordered pits, and no cell-contents (**tracheidal** or “transfusion-tissue”): they form lateral extensions from the margins of the xylem
 - iii. **Albuminous cells**, with dense protoplasm, forming lateral extensions from the margins of the phloem.
6. The two central **vascular bundles**, the constituents of which resemble those of the stem. Note that the xylem is directed towards the upper surface. Thick-walled sclerenchyma is scattered irregularly round and especially between the bundles.

THE ROOT.

I. Cut transverse sections of a young primary root of the seedling of *Pinus* or any young rootlet will serve. Treat with potash, or eau de javelle, and mount in glycerine. Observe—

1. The single superficial **piliferous** layer bearing root-hairs

2. The thick parenchymatous band of **cortex**

3. The **endodermis**, a single layer of cells having the characteristic marking on the radial walls (compare p. 94, 3). Within this lies—

4. The **stele**, which consists of—

a. The **pericycle**, a band three or four layers of cells in thickness.

b. Two to six Y-shaped groups of **primary xylem**, the fork of the Y directed outwards: between the limbs of the fork of each lies a **resin-passage**.

c. Groups of **primary phloem**, equal in number to the xylem groups, and alternating with them.

d. **Conjunctive parenchyma**, intervening between the xylem and the phloem, and sometimes forming a central mass of pith.

II. Cut transverse sections of a root of a Pine-tree, about $\frac{1}{16}$ th of an inch in diameter, which will show results of secondary thickening. Mount some sections in glycerine, others in chlorzinc-iodine. Observe:—

1. The absence of **cortex**, which will have been completely

thrown off at the endodermis, and the section thus consists only of the products of the stele

2 A peripheral band of **cork**, limited internally by a cork cambium, which has arisen in the outer region of the pericycle. Internally will be found the remainder of the pericycle in a quiescent state

3 The **phloem**, forming, according to the age of the root, a more or less complete ring. The constituents resemble those of the phloem of the stem, and the outermost may be crushed by pressure of growth from within

4 The **cambium**, as in the stem. It arises as in roots of Dicotyledons (see Fig. 7, p. 98)

5 The **xylem**, near the centre of which may still be recognized—

a The groups of **primary xylem**, arranged in the form of a Y, each having, as before, a resin passage in the fork

b The masses of **secondary xylem**, more or less fan shaped, and alternating in position with the primary xylem. The constituents of the secondary xylem resemble those of the stem. In old roots the xylem will have formed a complete ring, and annual rings may be observed

REPRODUCTIVE ORGANS

It has been above noted (p 154) that at the apex of the ordinary vegetative branch in spring there is an apical bud surrounded by a number of lateral buds, all of which normally develop into vegetative axes of the type above described. The reproductive organs of *Pinus* are produced on buds corresponding in position to these they are easily distinguishable, even at an early stage of development, with the naked eye, as male and female, which are borne on separate axes (diclinous), but may be upon the same tree. The following observations should be made upon specimens preserved in alcohol, otherwise they could only be made at intervals, according to the period of development of the organs in question.

A. Male inflorescence.—Note that the inflorescence while young appears as a bud covered with brown **scale-leaves**, in the axils of which are **lateral axes** easily seen on removing the scales. Of these lateral axes—

a. Those nearest the apex of the bud develop as lateral foliage-shoots, as is the case on the ordinary vegetative axis.

b. Below these, a number bear, in place of the two foliage-leaves of each spur, numerous **stamens**: to each one of these axes the term **flower** may be applied.

1. Separate a single male flower, and cut it longitudinally in a median plane: it will be found to consist of—

1. An **axis**, which bears—

2. At the base of it several small **scale-leaves**.

3. A number of **stamens** are inserted above.

Detach some of these stamens with a needle each consists of—

a. A short stalk, or **filament**, which bears at its apex—

b. An expanded **anther**, with two swellings on the lower surface (**pollen-sacs**, or **microsporangia**).

•II. Cut longitudinal sections of the male flower in which the pollen is not yet ripe, and mount in glycerine · examine with a low power. Note the arrangement of the parts as above described · the wall of each pollen-sac consists of a single layer of cells · in the pollen-sacs note the **pollen-grains** (**microspores**).

III. Mount ripe pollen-grains (*c* such as may be collected by shaking a male branch in June) in dilute glycerine, having previously wetted them with alcohol · Observe—

1. The two large lateral **wings**, usually filled with air, which facilitate the transfer of the pollen by the wind : these are produced by separation of the outer from the inner layer of the wall, forming a “blister” between them.

2. The central body of the pollen-grain, consisting of—

a A large cell, which constitutes the **greater** part of the grain, and from which the pollen-tube springs.

b A series of one or more smaller cells affixed laterally to the wall of the pollen-grain at a point between the wings · they are placed on the convex side of the grain, which is not so completely covered by the wings. These take no direct part in the formation of the pollen-tube

To see the internal structure of the pollen-grain, in alcohol material, treat with methyl-green or iodine, or better, with chloral hydrate and iodine. The number of the cells visible may vary according to the age of the pollen.

B. **Female branches or cones**.—Observe on a Scotch Fir, towards the end of June, that there are cones to be found in three different stages of development, the position of which is constant

a. **Small green cones**, one or more of which occur close to the apex of the shoot of the current year. Note that the basal part, or stalk, bears brown membranous scales, while the upper part is globular, and is marked out into numerous square areas, which are the apices of the **ovuliferous scales**. Comparing a shoot, which bears such young cones, with an ordinary vegetative shoot, it will be seen that the cones correspond in

position to the lateral buds, of which they are the morphological equivalent.

b **Larger green succulent cones**, which occur laterally at the apical part of the shoot of the previous year the arrangement of parts on these corresponds to that on (*a*).

c. Cones larger than (*b*), brown and with lignified tissues on these the scales are usually more or less separated from one another, so as to disclose the **seeds**, two of which are borne at the base of each of the ovuliferous scales. These ripe cones are seated laterally near the apex of the third annual increment of growth from the tip of the shoot.

1. Cut median longitudinal sections of a cone corresponding to stage (*a*). It should previously have been hardened with alcohol for some days. mount in glycerine, and examine with a low power Observe—

1. The central **axis**, not differing essentially from the young vegetative axis on this are borne scales of two orders easily distinguished by their size.

2. The **smaller** of these are the **bract-scales**, leaves borne by the axis of the cone, and the morphological equivalents of the brown scale-leaves which cover the winter buds

3. In the axil of each of these is borne one of the larger or **ovuliferous scales**, which are longer and more bulky than the bract-scales they alone can be seen externally On the upper surface of each of these, close to the axis, are borne—

4. Two **ovules** or **megasporeangia**, which are so placed that the micropyle is directed towards the base of the scale. if cut in a median plane, each ovule will be seen to consist of—

i. One **integument**, several layers of cells in thickness, with a widely open **micropyle** facing the axis this surrounds—

ii. The **nucellus**, a mass of parenchyma, near the centre of which is—

iii. The **embryo-sac** or **megaspore**, a cell much larger than those of the surrounding tissue, and lying some distance below the apex of the nucellus.

Pollen-grains may often be found seated on the apex of the nucellus: one or more of these may throw out **pollen-tubes**, which penetrate into its tissue.

Dissect off one whole ovuliferous scale, and observe on its upper surface, close to the base, **two ovules**. Note also the relative positions of the two sets of scales.

II. Take cones of the stage above described as (*b*). The material should be collected about the middle of June, and must be hardened in alcohol.

Strip off the ovuliferous scales of such cones. the ovules will remain adherent to the base of each. Cut longitudinal sections of the scales so as to pass through the median planes of the ovules: mount in pure glycerine, and examine with a low power. Observe—

1. The structure of the **ovuliferous scale**, which is traversed by vascular bundles, and resin-passages.

2. The **ovule**, which is united with the scale, and consists, as in the younger stage, of—

- a* An external **integument**: note the wide **micropyle**.

- b*. The **nucellus** as before, but larger.

- c*. The **embryo-sac**, filled with the thin-walled tissue of the **endosperm**. All the parts of the ovule are larger than in the younger stage, but retain the same relative positions. Note carefully that **pollen-grains** (one or more) are usually to be found lying on the apex of the nucellus, and that from the larger cell of each of them arises a cylindrical **pollen-tube**, which traverses the tissue of the nucellus as far as the apex of the endosperm, where it widens out into a large sac.

Observe near the apex of the endosperm, and embedded in it, one or more large vacuolated protoplasmic bodies: these are the **egg-cells**, or **ova**. From the apex of each a small cell is cut off shortly before maturity: this is the **ventral canal cell**. Leading upwards from this (*i.e.* towards the micropyle) may be traced a narrow **neck** or channel, inclosed by smaller cells than those of the surrounding endosperm. The neck, ventral canal cell, and ovum, together form the **archegonium**.

III. Remove ovules from cones of the second year, taken and preserved in alcohol about August. Dissect off from them the now hardened **integument** or **seed-coat**: note within this the delicate remnant of the **nucellus**, which covers the mass of **endosperm**. Soak the latter in water, and dissect

from it with needles the numerous **embryos**, which lie in the central cavity of the endosperm. treat them with potash, and mount in dilute glycerine. Examine with a low power, and observe—

1. The **suspensors**, coiled filaments consisting of numerous transparent thin-walled cells. At the ends of the suspensors are borne—

2. The **embryos**: they are more or less elongated, almost cylindrical bodies. in some cases (only one as a rule in each seed) they may have already formed—

a. An **apical cone**, which terminates the free, anterior end of the embryo; this being surrounded by—

b. A whorl of **cotyledons** of variable number.

c. The apex of the **radicle**, directed towards the suspensor (*i. e.* towards the micropyle of the ovule), and embedded in the tissue at the posterior end of the embryo.

Note that there is no definite boundary between the suspensor and the embryo. Also that though **polyembryony** is the rule—that is, a number of embryos are at first formed simultaneously—one of these supersedes the rest, and that one alone becomes differentiated as above described.

Ripe Seed.

Examine the ripe seed of *P. sylvestris*, and note the external hard and thick **seed-coat**: within this the **endosperm**, which incloses the single **embryo**. It has numerous **cotyledons**, and a **radicle**, the apex of which is directed towards the micropyle.

Germination.

Compare plants in different stages of germination, and observe the following points in the process:—

1. The endosperm swells, and bursts the testa.
2. The radicle protrudes, and curves downwards.
- * 3. The cotyledons elongate, and push out the stem and their own basal portion from the cavity of the endosperm.

4 The rest of the seed is usually carried upwards on the apex of the cotyledons, which, with the hypocotyledonary stem, clongate greatly.

5. The plumule develops, forming numerous acicular leaves, borne directly on the axis. It is only on the more advanced stem that axillary foliage spurs appear. Note that the cotyledons turn green while still protected from the light, below the soil, and within the testa.

PTERIDOPHYTA

A —LYCOPODINÆ

Heterosporous Type

SELAGINELLA MARTENSII

SPOROPHYTE

1. THIS plant is commonly grown in green-houses, and specimens can be readily obtained from nurserymen. In a well-grown plant note with the naked eye the following external characters :—

1. The **stem** ascending, frequently branched, apparently in a dichotomous, but really in a monopodial manner : the branching occurs only in a single plane.

2. The **leaves**, of small size, and simple in form, with a ciliate margin, and arranged in alternating pairs. each pair consists of a dorsal and a ventral leaf, the whole series thus forming **four orthostichies** : note the two different sizes of leaves—

a. The **larger ventral** leaves, arranged in two orthostichies, and without terminal awns.

b. The **smaller dorsal** leaves, also arranged in two orthostichies, each leaf being terminated by a fine **awn**.

Each leaf has a single central vein or **midrib**. Turn back one of the leaves, and observe ~~with~~ with a lens the small scale-like body called the **ligule**.

3. The **rhizophores**, long cylindrical branched organs, which arise at the points of branching of the obliquely ascending stem, and grow vertically downwards : note their frequent bifurcations.

Remove a rhizophore, which has grown down so as to reach the soil, and wash it : observe—

4. The delicate **roots**, which rise at the point where the rhizophores enter the soil, and branch in a monopodial manner ; and though they often seem to bifurcate it appears not to be a case of true dichotomy

Observe further that many of the branches of the stem may have a symmetrical arrangement of the leaves close to the apex : these are the fertile branches or **strobili**, which bear the **sporangia** : note that on these strobili or cones—

i. The leaves are all similar to one another and of small size

ii. That they are arranged in four symmetrical orthostichies.

iii. That, on turning the leaves back, one **sporangium** will be disclosed in each case. On comparing a number of sporangia which have been exposed in this way, it may be seen that there are two sorts of them—

a. **Megasporangia**, which are of a green or light-brown colour, and appear to be of rounded tetrahedral form.

b. **Microsporangia**, which are more nearly spherical, and of a reddish-brown colour

Note in older cones that the sporangia are already open, **dehiscence** having taken place in a plane parallel to that of the leaf.

Compare the small native species *S. spinosa*, found in mountain districts : its shoot is not dorsiventral, but radial, and all its leaves alike : rhizophores are absent. but in the strobilus it resembles *S. Martensii*.

II. Cut out as thick a piece of the stem as can be found, and about one inch in length : note a central white dot on the transversely cut surface : this is the central cylinder, or **stele**. Slice off the upper surface of the stem with a razor till the whole course of the stele is laid bare and observe with a lens—

1. The course of the stele, which is directly longitudinal and median.

2. The small vascular strands, which pass from the stele, without branching, into the leaves, and traverse the midribs of the leaves.

III. Cut transverse sections of a well-developed stem mount some in glycerine, others in chlor-zinc-iodine others again may be mounted in acid solution of aniline sulphate. Examine first under a low power, using a high power when necessary, and observe the following tissues in succession, starting from the periphery of the section :—

1. At the periphery a layer of small, thick-walled cells, forming an ill-defined **epidermis**, with no stomata. It is covered externally by a continuous **cuticle**. Beneath the epidermis, and not clearly marked off from it, is—

2. The **cortical tissue**: the cells of the peripheral part of it have thick stratified and lignified walls, with no intercellular spaces. Passing inwards there is seen a gradual decrease in thickness of the walls, and increase in size of the cells, till an abrupt limit is reached at—

3. The **lacunar tissue**, consisting of thin-walled cells, which form irregular **trabeculae** traversing the intercellular cavity in a radial direction. The innermost cells of these trabeculae are distinguished by a cutinised band on their radial walls, and although they are laterally separated from one another they represent the **endodermis**.

4. The **stele** is suspended by these trabeculae in the middle of the large air-cavity. It is of elliptical outline, as seen in the transverse section, and is composed of the following tissues.—

a. The **pericycle**, an irregular band of comparatively large, thin-walled cells, which completely surround the central tissues, and abut externally on the air-cavity, and the trabeculae. The cells of this layer, in common with all the outer tissues, including the epidermis, may contain chlorophyll-corpuscles. The pericycle near the apex is seen to arise from the division of a cell-layer, from which also the endodermis originates: as regards origin, therefore, it is not strictly comparable with the pericycle of Phanerogams.

b. The **phloem**, recognized as a tissue with thin cellulose walls, small cavities, and scanty contents: though reduced in bulk at the poles of the elliptical stele, it forms a band surrounding—

c. The **central xylem**, which appears as a spindle-shaped

mass of tissue when seen in transverse section, and consists of elements with lignified walls, and no cell-contents.

Small vascular strands of rounded outline, as seen in the transverse section, may be found opposite or near to the ends of the spindle-like stele; these are the leaf-traces, cut through on their course inwards from the leaves.

Note with a higher power—

1. The general appearance of the **phloem**, with its highly refractive cellulose walls.

2. Between this and the xylem is a somewhat irregular series of cells of the **conjunctive parenchyma**, with thin cellulose walls and plentiful protoplasm.

3. The chief constituents of the **xylem** are large prismatic **tracheides**, with peculiarly marked, lignified walls. There is no **parenchyma** in the xylem.

4. At the poles of the spindle-shaped xylem note tracheides of smaller size: these compose the first-formed **protoxylem**: the development of the xylem thus starts from the periphery, and proceeds towards the centre.

IV. Cut transverse sections of fresh leaves held in a piece of pith: mount in water or weak glycerine, and observe—

1. The **epidermis** of the upper surface consists of conical cells, each of which contains a single large **chlorophyll corpuscle**. Stomata are absent.

2. Beneath this is the **spongy parenchyma**, which incloses centrally—

3. A single **vascular strand**.

4. The **epidermis** of the lower surface consists of smaller cells containing chlorophyll, and with **stomata** opposite the midrib.

V. Cut transverse sections of the rhizophore: observe that it contains a single central stele, composed of one group of xylem, and one group of phloem. There is but one protoxylem group; the stele is thus **monarch**. There is no lacunar tissue, but a normal endodermis. The stele of the root resembles that of the rhizophore.

VI. Cut longitudinal sections through fertile branches similar to those cut from the vegetative bud, and examine them under a low power. In the lower part, if the section was **median**, the

same succession of tissues may be recognized, as has been already described in the transverse section (III). Starting from the outside, they will be as follows --

1 **Epidermis** } these are hardly to be distinguished one
2. **Outer cortex** } from another the cells of both are pros-
enchymatous, and thick-walled, and show a gradual transition
to—

3 The **inner cortex**, in which the walls are thinner, and the form of the cells parenchymatous.

4. The **lacunar tissue**, the cells of which are elongated in a radial direction.

5 The **pericycle**, consisting of elongated parenchymatous cells, with cellulose walls, and often containing chlorophyll.

6 The **phloem**, the most prominent elements of which are long narrow elements with cellulose walls and sparing contents. these are the **sieve-tubes**.

7 The **xylem**, the most prominent elements of which are **spiral** and **scalariform tracheides**, similar to those to be described below as composing the xylem in the Feins

Observe the general arrangement of the stem, leaves, and ligules.

In the lower part of the sections a mature **sporangium** may be found in the axil of each leaf. The sporangium may have lost its spores partially or entirely during the preparation of the sections. It will consist of--

a A short massive **stalk**.

b A **wall** inclosing the central cavity the wall will be found under a high power to consist of three layers of cells --

i. The outer, consisting of thick-walled cells, more or less elongated radially.

ii A layer of small, compressed cells.

iii. A layer of thin-walled cells, elongated radially. this is the **tapetum**, which is here persistent until the spores are ripe

Surrounded by the wall will be found--

c. **Spores** of two sorts, contained in different sporangia --

i. **Microspores** of relatively small size. these will be found in large numbers in certain sporangia, which will accordingly be recognized as **microsporangia**. When ripe they may be still

seen to cohere in groups of four: each spore is a single cell with a brown wall.

11. **Megaspores** of relatively large size: **four** only of these will be found inclosed in a single sporangium, which is accordingly termed a **megasporangium**. Each spore consists of a thick wall, with numerous external projections, surrounding a large cavity filled with protoplasm, &c.

THE GAMETOPHYTE OR OOPHYTE

VII. Spores of both kinds may be obtained free by drying branches which bear sporangia on sheets of paper. Pick out the **megaspores**, and mount them in olive-oil: dissect off the brittle outer coat of the spore with needles, and examine under a high power. It will be seen that the chief contents of the ripe spore are a protoplasmic matrix inclosing oil-globules and aleurone grains, while traces of the cells of the **prothallus** may be recognized even in these preparations.

If plenty of spores are to be had, embed a quantity of them, and cut sections, mounting them in glycerine. Observe—

1. The character of the wall, consisting of—

a. An outer thick, yellow **exospore**.

b. An inner thin **endospore**.

2. The contents as above described: the natural position of the cellular tissue of the **prothallus** may be seen to be at the apex of the cavity of the spore.

VIII. Spores of both kinds should be collected in considerable quantity by drying on paper, and then be sown on moist soil or sand, and left to germinate. From the microspores motile spermatoids are produced, which fertilize the archegonia borne on the prothallus of the megaspore but it cannot be expected that elementary students will follow the details of this process. As a consequence of fertilization of the female prothallus, in a few weeks young seedlings will be seen with an erect axis, bearing small leaves. Note that the axis of the seedling branches at an early period.

Remove some of these seedlings from the soil, and note the

monopodial branching of the root, and the megaspore still attached laterally to the axis.

Longitudinal sections should be made through the young seedling, so as to traverse also the megaspore attached to it in such sections it will be readily seen that a lateral outgrowth (the **foot**) projects from the base of the axis into the cavity of the megaspore: also that the latter is filled with a cellular tissue of the prothallus, from which the nutritive substances above noted in the mature megaspore will have been removed.

A detailed description of work to be done on *Lycopodium clavatum*, as a homosporous Lycopod, is given in the larger edition.

B.—FILICINEÆ

I. Homosporous Type

NEPHRODIUM FILIX-MAS (The Male Shield Fern)

A.—MATURE SPOROPHYTE

I.—External Characters

I. **TAKING** a well-grown plant of the common Male Fern in summer, wash the soil away from the roots, and observe the following external characters .—

A. The **stem** is oblique and ascending · it is **not** branched at its apex : its surface is covered by the persistent bases of the **leaves**, which are densely covered by numerous brown scaly **hairs** (**paleæ** or **ramenta**)

B. The **leaves**, the most prominent of which are—

i. The fully developed green leaves of the current year : of these the following parts may be recognized :—

a. A long, almost cylindrical leaf-stalk, which is traversed by two longitudinal, lateral ridges or reduced wings. This leaf-stalk supports—

b. The numerous **pinnæ**, which are arranged in two lateral rows, corresponding in position to the lateral ridges above mentioned : note that the arrangement of the veins in the segments of the pinnæ is based upon repeated bifurcation of the stronger veins. On the under side of the pinnæ will frequently be found—

c. **Sori**, which are roundish brown groups of small stalked bodies (**sporangia**), each sorus being covered by a kidney-shaped **indusium**.

ii. The bases of the leaves of previous years will be seen, covering the lower part of the stock or stem externally. observe that lateral **buds** are frequently to be found connected with these, being attached to their ab-axial side, near to their point of junction with the stem.

iii. Nearer the apex of the stem than the expanded leaves of the current year, and completely covering it, are **young leaves**, densely covered with ramenta. these, together with the axis, constitute the **apical bud**. Note that the apex of each such leaf is rolled up like a crozier (**circinate vernation**).

C The **roots** are rather thin and brown, with transparent apices they are inserted on the stem at a point close to the insertion of the leaf-base. The branching of the roots is **monopodial**, and their branches appear in acropetal succession.

II—*Anatomical Characters to be observed with the naked eye*

II Having observed the above external characters, remove the roots, **keeping** the transparent apices of the young roots, as well as the thickest parts of the old roots: these should be preserved in alcohol for further treatment.

III Remove from the apical bud the large quantities of **scaly** hairs (ramenta), so as to lay bare—

- 1 The **young leaves**, with their circinate vernation.
- 2 The broad **apex of the stem**, with leaves in various stages of development around it
- 3 The **young roots**, which will be found already present, close to the bases of the young leaves.
- 4 The **young buds**, which may be observed at a very early stage on the ab-axial side of the leaves

If the specimen be a large one, with a stock 5 or 6 inches in length, cut off about 2 inches of the older end of the stem exposed as above, and boil it in dilute hydrochloric acid till the parenchyma is soft for further treatment of this see below. Meanwhile smooth the cut end of the remainder of the stock with a razor, so that it may **present** an even surface of transverse section, and observe—

a. The great irregularity of outline, due to the close crowding of the bases of the leaves.

b. The dark brown band of **sclerenchyma** bordering the periphery of the section.

c. The great bulk of the stem, consisting of yellowish **parenchymatous ground-tissue**.

d. A number of larger vascular strands, **steles**, embedded in the ground-tissue, forming an irregular ring

e. A number of smaller vascular strands, outside this ring, which run out into the petioles.

This stem is thus **polystelic**, and it will be seen below that each stele corresponds in essential structure with the single stele of the monostelic *Selaginella* above described

IV. Divide the stock, including the apical bud, into two symmetrical halves by cutting it in a median longitudinal plane: smooth one of the cut surfaces with a razor, and observe—

a. That the stem is of almost equal thickness throughout its length, *i.e.* it is roughly cylindrical.

b. That its external conformation is very irregular by reason of the closely crowded insertion of the leaves.

c. The ground-tissue as before

d. The large **steles** (*d*, above), which are not continuous in direct longitudinal lines, but form an interrupted series

e. The smaller strands of the **leaf-trace** (*e*, above), which in some cases may be followed, after a little careful dissection of the parenchyma which surrounds them, from one of the larger steles of the central system into the base of one of the leaves.

Slice away carefully the external tissues of the posterior part of the stock, so as to lay bare the central system of larger steles: it will then be seen that these form a continuous **network** with large meshes, and that each mesh is opposite the point of insertion of one of the leaves, hence it is called a **foliar gap**. Observe also that the several strands, which pass out into any individual leaf, are given off from the margin of its own mesh.

Confirm these observations by the dissection of the stock:

the parenchyma may be easily removed, leaving the vascular system as a network, which gives off numerous weaker strands from the margins of its meshes ; these weaker strands run out into the leaves.



FIG. 28.—Vascular skeleton prepared by maceration and dissection from the stem of *Nephrodium Filix-mas* (After Remke) (2.1)

By careful dissection a skeleton may be prepared similar to that shown in Fig. 28.

III.—*Microscopic Investigation*

V. Cut transverse sections of the stock of *Nephrodium* : it is hardly to be expected that a transverse section of so bulky a stem as this could be cut so uniformly thin that the structure of all the tissues could be well seen : it is better therefore to cut a

number of sections, each extending over a comparatively small area, and to study the various tissues separately. Mount some in glycerine or glycerine jelly, others in chlor-zinc-iodine. Examine under a low power, and observe successively the following tissues, starting from the periphery of the stem —

a. An **epidermis**, consisting of a single, somewhat irregular and ill-defined layer of cells, with dark brown outer walls. Their arrangement is disturbed at the point of insertion of the **ramenta**, which appear as plates one layer of cells in thickness, rising obliquely from the epidermis. Beneath this is—

b. The **ground-tissue**, which is differentiated as—

i. An outer narrow band of tissue, with rather thick, colourless, pitted walls, and cell-contents with much starch. There are no intercellular spaces.

ii. A band of **sclerenchyma**, with thick, yellow, obviously stratified, and pitted walls, cell-contents as in (i), and no intercellular spaces. This merges gradually into—

iii. The bulky central mass of ground-tissue, in which the steles are embedded. It consists of cells with comparatively thin, pitted, cellulose walls, protoplasmic contents with much starch, and with intercellular spaces. **Internal glandular hairs** are also found in the intercellular spaces.

c. The **steles** of elliptical outline. They are embedded in the ground-tissue, and are sharply circumscribed by a narrow, light brown layer of cells without intercellular spaces. This is the **endodermis**. Among the tissues inclosed by this sheath, note that a large central mass may be distinguished as consisting for the most part of elements with large cavity, no cell-contents, and rather thick walls with a peculiar marking. This is the **xylem**. Between this and the endodermis is a broad band of tissue with thin, bright-looking walls, and with protoplasmic contents: this is the **phloem** and **pericycle**.

In the sections treated with chlor-zinc-iodine note that the walls of the inner ground-tissue stain blue, and that starch is found in the cells; that the endodermis appears browner than before; that the walls of the phloem stain blue (cellulose), and the contents yellowish, while the pericycle, which lies outside it, may be distinguished from it by the starchy contents of its

cells: the walls of the chief constituents of the xylem stain yellow (lignified).

VI. Since it is easier to prepare good sections from the rhizome of *Pteris aquilina* (the Common Bracken) than from the stock of *Nephrodium*, and at the same time its steles are larger, and their elements more distinct, it will be found more convenient to continue the high power observation of the stele in the former plant. The structure is essentially similar in both cases, and only differs in detail.

Cut thin sections from the rhizome of *Pteris* mount some in glycerine, others in chloro-zinc-iodine. or these sections may be stained with hæmatoxylin and mounted in Canada balsam.

1 Observe with a lens, or with the naked eye, that in this case there are two concentric rings of steles present. The outer series consists of a number of relatively small steles, of which the one opposite the lower surface is usually the largest. This series represents the typical ring of steles as found in *Nephrodium*. The central series of steles may be regarded as an additional complication derived from the outer series: it consists usually of two or three separate steles of larger size, but occasionally they may be connected by one of their margins, or even by both. in the latter case they form a closed ring.

2 The **sclerenchyma** of the stem consists of a peripheral dark brown band similar to that in *Nephrodium*, outside which is a scarcely distinguishable epidermis. An additional incomplete ring of similar elements lies between the two series of steles. it is usually composed of an upper strongly curved portion, and a smaller flat portion which is nearer the lower surface. Small isolated patches of brown sclerenchyma may also be seen dotted about in the ground-tissue.

3 Examine also the parenchymatous ground-tissue, which will be found to consist of cells with thin cellulose walls, and mucilaginous protoplasmic contents, with much starch. Note especially small pegs and rods, which appear as superficial outgrowths from the walls adjoining the intercellular spaces.

Select a stele of medium size, in thin section, for detailed observation under high power. Starting from its periphery, observe—

i. The **endodermis**, which is a definite but narrow layer of cells, without intercellular spaces between them, and with dense brown contents.

ii. The **pericycle**, a layer one or sometimes two cells thick, lying immediately within the endodermis. Its cells have cellulose walls, and protoplasmic contents: they also contain numerous starch-grains. Here again, as in *Selaginella*, both endodermis and pericycle arise from divisions in a common layer of mother-cells.

iii. Within this is a band of **phloem**, which is wider at the flattened sides of the stele, but narrower at the two ends. The first-formed elements of the phloem, or **protophloem**, form a distinct zone towards the outside: they are angular and flattened in appearance, with somewhat swollen cellulose walls. The rest of the phloem (metaphloem) consists of—

a. **Sieve-tubes**, which may be recognized by their polygonal outline, large cavity, sparing contents, and cellulose walls: note where two sieve-tubes are contiguous that bright yellow granules may be seen adhering to the wall: these indicate the position of the **sieve-plates**.

b. The **phloem-parenchyma**, cells with thin cellulose walls, and dense protoplasmic contents, but without starch.

iv. An elliptical area of **xylem** occupies the centre of the stele. It consists of two kinds of elements:—

a. **Tracheides**, large circular or polygonal elements with thick lignified walls, and no cell-contents. The walls show a peculiar structure, which will be better understood on comparison with their appearance in longitudinal sections. At certain points in the xylem-mass the tracheides are seen to be of smaller size, and to have thinner walls, which are sometimes crushed out of shape. these are the **protoxylem groups**, and because they occur within the substance of the xylem, they are said to be **mesarch**.

b. **Xylem-parenchyma**, cells with cellulose walls, and protoplasmic contents, and also starch. They are scattered irregularly

among the tracheides, and also form a layer surrounding the xylem, and separating it from the phloem.

VII. Longitudinal sections should also be cut, so as to traverse both series of steles: they may be treated as above directed. Note in these the thin-walled **ground parenchyma**, and the brown **sclerenchyma**: the latter consists of fibrous cells, with peculiar crossed pits in their walls.

i. The **endodermis** consists of narrow oblong cells with square ends, and with brown contents.

ii. The **pericycle** is composed of wider oblong cells, with protoplasmic contents, and starch. the ends are square or oblong.

iii. The **sieve-tubes** which appear as wide tubes, with pointed ends, and cellulose walls. the lateral and terminal surfaces of the walls which separate contiguous sieve-tubes are covered with numerous **sieve-plates** (best seen in sections stained with chlor-zinc-iodine), to which round, highly refractive granules adhere: these granules stain yellow with chlor-zinc-iodine. In surface view it is seen that the sieve-plates are so large, relatively to the area of the wall between them, that the latter has the appearance of a coarse reticulation.

Note especially the irregular beaded outline of the wall in longitudinal section: this will be best seen in sections which have been cut so as to pass through the phloem in a plane parallel to the flattened side of one of the larger steles.

iv The **scalariform tracheides**, which are the main constituents of the xylem: being elongated and pointed, while the walls are marked by transversely-extended bordered pits, arranged regularly so as to give a ladder-like appearance; but they differ from them in one point, for by a careful examination of fine sections it may be ascertained that the pit-membrane which remains permanently in *Nephrodium*, is often broken down on the oblique terminal walls in *Pteris*: this is, however, exceptional for Ferns.

v. The **conjunctive parenchyma**, distributed among both sieve-tubes and tracheides.

* VIII. Separate out some pieces of the stele from the stem of *Pteris* or of *Nephrodium*: clear away the surrounding tissues

from them, and warm them gently in a test-tube with a little potassium chlorate and nitric acid (see Schulze's macerating fluid, Appendix A.), till the elements of the bundle may be separated easily one from another. then stop the action by diluting with water, and mount in water or glycerine. By preparing them in this way the tracheides, &c., may be subjected to separate examination, and their form and structure may be more exactly made out.

Apply the same process to the sclerenchyma, and observe the form and marking of the walls of its constituent elements.

IX. From around the apical bud of a well-grown plant of the *Nephrodium* remove successively the bases of the leaves of previous years, those of the current year, and finally the larger circinate leaves, which would have unfolded in the following year. Carefully remove the smaller ones with a scalpel, and then with forceps gradually pull off the large mass of brown scales, which completely cover the extreme apex. With a stiff camel's-hair brush remove the rest of these scales, including the youngest of them, which will still remain round the *punctum vegetationis*: after this treatment it will be easy to observe with a pocket lens—

1. The **apical cone** (*punctum vegetationis*), a rounded papilla, occupying a central and terminal position in the flattened apical region.

2. The **young leaves**, situated round the apical cone, and successively larger the further they are from the apex. Note the circinate curvature which appears at an early period in their development.

X With a sharp razor, wet with water, or with very weak spirit if the material be fresh, or with strong spirit if it has been previously hardened in alcohol, remove the extreme apex of the *punctum vegetationis*, taking care to cut accurately in a transverse plane: mount in water or in weak glycerine, and examine with a low power. If the section be thin enough, it will be seen that a large cell of **triangular outline** occupies the centre of the apical cone, while the cells immediately surrounding it are arranged in more regular order than those at a greater distance. This cell is the **apical cell**, and the cells

surrounding it have been derived by cell-division from it, by means of walls parallel to its three sides they are called the **segments**, and it may readily be seen that these again undergo subdivision. If the section be not sufficiently transparent, it may be treated with very dilute potash and weak glycerine, or, better, with "eau de javelle," which will clarify the tissues, and make the cell-walls more distinct.

The form of the apical cell, and of the segmental cells which

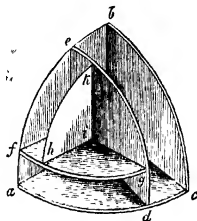


FIG. 29.—View of a model of a three-sided pyramidal (or tetrahedral) apical cell, as seen from above, the walls *d i*, *f g*, *h i*, denote successive walls by which segments have been cut off from the growing apical cell. *i* is the apex of the pyramidal cell, at which point the three youngest segmental walls cut one another. (After Sachs.)

surround it, will be readily appreciated on comparison of fig. 29.

XI. From the apex of another plant cut median longitudinal sections: mount in weak glycerine: a very little dilute potash may be added if the sections are not transparent enough, or they may be treated first with "eau de javelle," and then be mounted in glycerine.

If any one of the sections has passed through the apical cone, in a median plane, the **apical cell** will be seen presenting a wedge-like appearance, and the cells around it will show, in the regularity of their arrangement, that they have been derived from segments successively cut off from the apical cell. (Compare Fig. 30.) It may be concluded from the observation

of transverse and median longitudinal sections that the form of the apical cell is that of a three-sided pyramid.

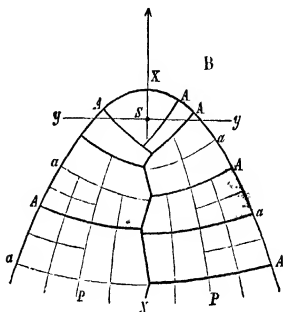


FIG. 30 — Diagram showing the arrangement of cell-walls as seen in a median longitudinal section through an apical cone of the stem with a pyramidal apical cell. *A, A*, are the segmental walls, which form part of the system of anticlinals, *a, a*, walls by which each segment is cut into two equal halves: these complete the anticlinal curves; *P, P*, periclinals, which are not completed up to the apex (After Sachs)

The structure and mode of origin of the young leaves should also be observed in the median longitudinal sections.

The Root.

XII. Cut transverse sections from the root of *Nephrodium*, selecting for that purpose the thickest part of an old root mount in glycerine, and observe under a low power. There is a single central **stele**, surrounded by a broad belt of **cortex**. Put on the high power, and note at the periphery of the cortex—

1. The **pubescent layer**: certain cells of this superficial layer have grown out as **root-hairs**, remnants of which may still be seen.

2. The greater part of the section consists of the bulky, brown-walled **cortex**, of which the outer parts are thin-walled; but

passing inwards there is a sudden increase in thickness of the wall, so as to form a dense sclerenchymatous ring. this surrounds—

3 The **endodermis**, which consists of a single layer of cells flattened tangentially, and having the usual dot-like marking of the radial walls : this may be difficult to observe, as the radial walls are often pressed out of shape. Within this layer lies—

4. The **pericycle**, which usually consists of two layers of cells with thin walls, and obvious protoplasmic contents.

5 The centre of the stele is occupied by a diametric plate of **xylem**, with a group of small elements, **protoxylem**, at either end. The stele is therefore **diarch**. The protoxylems are situated at the extreme periphery of the xylem, abutting upon the pericycle. they are therefore said to be **exarch**, and the xylem is developed towards the centre (centripetal), where the largest tracheides are to be found.

No xylem-parenchyma is to be found among the tracheides, but a sheath of parenchyma surrounds the xylem-strand as a whole, and separates it from—

6. The **phloem**, which forms two bands, one on either side of the xylem-plate. It shows exactly the same characteristics as that of the stem. In old roots crushed **protophloem** elements may be recognized towards the periphery of the phloem-strands.

XIII. Cut median longitudinal sections of the apex of a root which has been hardened in alcohol. at most only one absolutely median section can be obtained from a single root. it will be found convenient to embed the apex of the root in paraffin, or to hold it between pieces of pith or carrot. Mount in glycerine, and examine first with a low power. choose out those sections in which there is a symmetrical arrangement of tissues around a single, large, tetrahedral **apical cell**, which lies at some distance from the extreme apex. (Compare Fig. 31.)
Note—

1. That the orientation of the apical cell is constant, *i.e.* one corner is directed towards the older part of the root, while the side opposite that corner, *i.e.* the anterior face of the cell, is perpendicular to the **axis of the root**.

2. That around the apical cell are regularly arranged **segmental cells**, which have successively been cut off from it by walls parallel to the sides of the apical cell. Of these —

a Those successively cut off from the base form the **root-cap**, dividing up by regularly arranged walls into a mass of regular cells.

b Those cut off from the sides of the apical cell form the body of the root: these also divide by walls in regular succession. Observe carefully the arrangement of these walls, and

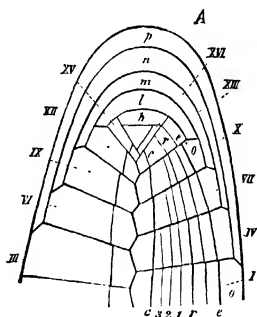


FIG. 31 —Diagram illustrating the arrangement of cell-walls as seen in a median longitudinal section through the apex of the root of a Fern. I, III, &c., indicate segments cut off from the sides of the apical cell, which go to form the body of the root, *h, l, m, n, p,* are successive segments from its base, which go to form the root-cap (After Goebel)

by comparison of several sections ascertain their order of succession, and their relation to the various tissues of the root above described.

XIV. Cut successive transverse sections of the apex of a root which has been hardened in alcohol: this may easily be done if the root be held between pieces of pith, or better by embedding in paraffin. If possible, keep all the sections in their proper order of succession, and mount in glycerine. Examine

with a low power, and choose out those in which the large apical cell is to be seen. Observe carefully—

1. The **form** of the **apical cell**, apparently **three-sided**: combining this result with that obtained by examination of the longitudinal sections, the form of the whole cell must be a **three-sided pyramid**. (Compare Fig 29)

2. The **segments** are arranged in regular order round it, and are cut off successively from the three sides.

3. Note the mode in which the several segments are further divided.

Next examine a section which has passed through the root-cap **immediately above the apical cell**: this will include the young segments cut off from the base of the apical cell by transverse walls, and destined to form the root-cap. Note the first divisions of these segments by walls arranged crosswise: it may be seen that these walls do not coincide in position in successive segments.

The Leaf

XV. Cut transverse sections of the petiole of *Nephrodium*: note a number of isolated **steles**, similar to those in the stem, but arranged in a horse-shoe-like curve, the heel of the horse-shoe being towards the adaxial side. There is a hypodermal zone of sclerenchyma, and the layer of ground-tissue next the endodermis has the tangential walls of its cells strongly thickened. The protoxylems are endarch, on the side of the xylem next to the organic centre of the section.

XVI. Cut transverse sections of a pinna of a leaf of *Nephrodium*, which has no sori upon it: mount in weak glycerine, and observe with a low power that the outline of the section shows the leaf to be of equal thickness throughout, except where traversed by vascular strands: at those points the pinna is thickened, the lower surface projecting convexly.

Examine with a high power, and observe successively the following tissues, starting from the upper surface:—

1. A regular **epidermis** with a thin cuticle: the epidermal cells contain chlorophyll: there are no stomata.

2. The **mesophyll** consists in its upper part of thin-walled cells containing chlorophyll, and with small intercellular spaces, this passes by gradual transition into the lower part, where the intercellular spaces are larger, and the form of the cells less regular. Internal glandular hairs are frequently to be found in the intercellular spaces of the mesophyll.

3 The lower **epidermis**, the cells of which also contain chlorophyll: numerous **stomata** are present: note the form of the two **guard-cells** as seen in transverse section, and their position in relation to the epidermis.

4 Here and there **vascular strands** of circular outline will be found embedded in the mesophyll the larger of these correspond in position to the swollen ribs of the pinna.

Note the **endodermis** as a continuous layer of cells, which completely surrounds the circular bundle, and within this the xylem and phloem elements, similar to those of the stem. The steles of the petiole, as they pass upwards from the petiole, lose the phloem on their inner side, and thus show an approach to the **collateral** type of vascular bundle, the xylem being nearest to the upper surface of the leaf.

XVII. Cut tangential sections (or strip off the epidermis from the upper and the lower surface of the leaf mount as before, and compare them.

a. The epidermis of the **upper surface** will be found to consist of cells with sinuous outline, and protoplasmic contents, with chlorophyll: **no stomata** will be found.

b. The epidermis of the **lower surface** consists of cells similar to the above: there are **stomata** with two guard-cells

The Sporangia.

XVIII. Having examined the sori as directed on p. 180 with the naked eye or with a lens, cut transverse sections through pinnae of leaves which bear **sori**, taking care that the sections shall pass through one or more of them: mount as before, and examine with a low power. Note—

1. The structure of the pinna, as above described.

2. Opposite to, and seated upon a vein will be found the

membranous **indusium**, which is seated upon the enlarged vein, or **placenta**, and, like an umbrella, covers over —

3 The **sporangia**, which are biconvex-lens-shaped, brown, stalked capsules, attached to the placenta, and filled with—

4 Numerous roundish, brown, unicellular **spores**.

Observe more closely the structure of the single sporangium. It is composed of—

i. The **stalk**, which is of considerable length, and usually consists of three rows of cells. Stalked glandular hairs are often found as lateral branches on the stalk of the sporangium in this species.

ii. The **capsule**, which has the form of a biconvex lens, and consists of a marginal series of cells with peculiarly thickened walls, which constitute the **ring**, or **annulus**; and thinner-walled, flattened cells, which together form the lateral walls of the completely closed sporangium.

Place a number of mature, but not yet ruptured sporangia upon a dry slide: warm them very gently over a spirit-lamp, and observe quickly under a low power. note the movements of the sporangia, so as to eject and scatter the spores. there is first a straightening of the annulus or ring, which causes rupture of the thin region of the wall. then a subsequent recovery, with a sudden jerk, to its original curved position. The spores are thus forcibly thrown out.

Similar results may be obtained by mounting in water, and subsequently adding glycerine; in fact, on the removal of water by evaporation into the air, or by a reagent such as glycerine, the curved annulus tends to straighten itself, and so ruptures the thin wall of the sporangium.

Note sporangia in which the thin lateral walls have been ruptured transversely, the ring having straightened itself out.

Examine single **spores** under a high power. they are unicellular bodies, having a brown wall, with external band-like outgrowths of the exospore, or outer layer of the wall. All the spores are alike (**homosporous**).

The various stages of development of the sporangium may be found in any sorus in which only the first sporangia have come to maturity. treat the sections previously with weak

potash : if this makes them too transparent, neutralize with weak acetic acid, and mount in glycerine ; or the sections may be treated at once with "eau de javelle," and then be mounted in glycerine.

B.—THE GAMETOPHYTE, OR OOPHYTE.

I Dry some of the leaves of *Nephrodium*, which bear sori, on a piece of paper. the spores will then be set free by the rupture of the sporangia, and they may thus be collected in large quantities. Sow some of them on damp earth : keep them moist, and sheltered from direct sunlight : they will then germinate, and after a few weeks the surface of the soil will be found to be covered with small, green, flattened bodies each of which is an individual **prothallus**.

From time to time, pick off some of the young prothalli with a needle from the surface of the soil : by this means a series of observations may be made which will illustrate successive stages of development of the prothallus.

II. Examine a single, fully-grown prothallus with the naked eye, and observe—

1. The **form**, which is flattened, and more or less kidney-shaped, with a depression of the margin, at the base of which is the **organic apex** of the prothallus. Note that the central part of the prothallus is often perceptibly thicker than the periphery : this thicker part is called the **cushion**.

2. The **position** of the prothallus while growing : it is usually oblique to the surface of the soil.

3. The **rhizoids**, which spring from the under surface of the cushion, and run downwards into the soil.

4. The **green colour**, due to the presence of chlorophyll : the prothallus is thus capable, under suitable circumstances, of carrying on the process of elaboration of fresh organic substances.

III. Wash a fresh, well-developed prothallus carefully in water, so as to remove the soil from the rhizoids : mount it whole in water, with the lower surface directed upwards, and examine it with a low power. Observe again the chief points

seen above with the naked eye, which are now more plainly visible, and note especially—

1. The **form** and **structure** of the cells in the lateral, thinner portions of the prothallus. they are polygonal, and have thin cellulose walls, and protoplasm containing a nucleus and numerous chlorophyll-corpuscles: the cells at the margin are often extended as hair-like outgrowths.

2. The cells composing the cushion are of similar structure, but are aggregated in a mass more than one layer of cells in thickness—many of the cells will be seen to have grown out as rhizoids.

3. The depressed **apex** of the prothallus, which is occupied, not by a single wedge-shaped cell, as is the case in early stages of development, but by a closely aggregated series of marginal cells, with thin cell-walls, and every appearance of recent and repeated cell-divisions.

4. The **antheridia**, which are hemispherical outgrowths, situated chiefly on the posterior and lateral portions of the under side of the prothallus.

5. The **archegonia**, which are situated on the cushion near to the organic apex of the prothallus—the multicellular **neck** of the archegonium projects from the surface of the prothallus as an elongated cylindrical structure.

Under the low power select one mature antheridium, and, without moving the slide, adjust the higher power so as to observe the structure of the same antheridium in detail. It will then be seen that it consists of—

a A **wall**, composed of a single layer of tabular cells: this completely surrounds—

b. The **spermatocytes**, or mother-cells of the spermatozoids, which are small, and not very numerous.

Other antheridia may be found which have already burst the outer wall: in these the contents of the mother-cells may perhaps be seen escaping from the ruptured antheridium as spiral **spermatozoids**, endowed with active movements. If a preparation showing motile spermatozoids be treated with a weak solution of iodine, the movements will cease with the death of the spermatozoids, which will assume a brown staining, while

the **cilia** attached to the anterior ends of them will then be clearly seen.

Select under the low power one mature **archegonium**, and then observe it in detail under the higher power. If the neck be vertical, which would under the circumstances be the natural position, since the prothallus was mounted with the lower surface uppermost, there will then be seen, on focussing down upon it, **four** rows of cells composing the wall of the neck, and surrounding one cell, the **canal-cell**.

IV. Harden some prothall with alcohol, or with picric acid and then with alcohol. The preparations described below may also be made from fresh material, but the results will not be nearly so good as if one of the above methods of fixing and hardening be adopted.

Hold a prothallus thus prepared between pieces of pith, or embed as directed on p. 8. then cut sections perpendicularly to the surface of the prothallus, so as to pass through the cushion, following the organic axis from base to apex. Mount in glycerine, and examine first with a low power.

The **lower surface** may easily be recognized by the presence of **rhizoids**: on this lower side, chiefly near to the apical end of the section, which is characterized by its small cells with **thin walls**, will be found **archegonia**: these may be recognized by the multicellular **neck**, which projects beyond the surface of the section. In some cases the canal of the neck may appear of a deep brown colour. this is the case in old archegonia which have not been fertilized, and they should be disregarded. Select one **archegonium** of full size and healthy appearance, and examine it under a high power.

Observe that it consists of—

A. The **central series** of three cells, which may be distinguished as—

a. The **canal-cell**; this is oblong in form, and its walls are subject to mucilaginous degeneration: it occupies the channel of the neck, and has been above alluded to as being visible when the neck of the archegonium is seen from above.

b. The small **ventral canal-cell**, which lies immediately below the oblong canal-cell, and is of rounded form.

c. The **oosphere**, or **ovum**, which is of relatively large size, and roughly spherical form : it is embedded in the tissue of the cushion, and consists of a dense mass of granular protoplasm.

B. The **neck**, which is composed of cells arranged in four rows, constituting together a cylinder or tube, one layer of cells in thickness. this projects from the surface of the prothallus, and incloses the cells (a) and (b) of the central series, while (c), the ovum, is embedded in, and surrounded by, cells of the cushion

At the end of the section more remote from the apex may be found **antheridia**. Select one fully developed, and it will be seen to consist essentially of an outer wall one layer of cells in thickness. this incloses a central mass of cells, the contents of which may be seen to be rounded off, and to have assumed the form of a closely coiled spiral. these are the **spermatocytes**, or mother-cells of the spermatozoids.

The dehiscence of the antheridia, the escape of the spermatozoids, and their movement, should be observed with particular attention in fresh prothalli mounted in water ; also the opening of the apex of the neck of the archegonium. in both cases the process depends upon a mucilaginous degeneration of cell-walls of the inner cells, and a subsequent swelling by taking up water, and consequent rupture of the outer walls. Further, the movements of the living spermatozoids may be followed, and the act of fertilization observed. the spermatozoids pass through the mass of mucilage which fills the neck of the archegonium, and finally coalesce with the ovum.

C.—THE YOUNG SPOROPHYTE, OR FERN-PLANT.

1. The result of the process of fertilization of the ovum by the spermatozoids is the development of a new Fern-plant (the **Sporophyte**), and in cultures which have been continued for some months such young Fern-plants may be seen attached to the prothalli, but one prothallus produces only one young Fern-plant.

Select a prothallus to which a young Fern-plant is thus

attached, and wash from it the soil which adheres to it. Examine it with a lens, and observe—

1. That the prothallus itself is similar in form and structure to those before observed.

2. That the young Fern-plant is firmly attached to its under surface by a lateral protrusion (**foot**).

3. That the young Fern-plant consists of the following parts :—

a. A **root**, which turns downward into the soil.

b. A lateral protrusion, the **foot**, which maintains a close physiological connection between the prothallus and the Fern-plant.

c. A first leaf, or **cotyledon**, with an elongated petiole, and bifurcating, expanded, upper part. this usually grows upwards through the depression at the apex of the prothallus.

d. Between the base of the cotyledon and the foot is the **apex of the stem**, which continues its growth, and produces new leaves.

A prothallus, bearing a young plant, should be cut in median longitudinal section, so as to traverse the insertion of the young plant, and its organs. Examine under a low power, and observe the physiological connection by means of the foot between the two generations : also the relative position of the parts of the embryo, as above noted.

BRYOPHYTA

A.—MUSCI

POLYTRICHUM COMMUNE, L.

A.—GENERAL EXTERNAL CHARACTERS

1. OBSERVE in well-grown specimens of this Moss taken in spring or early summer—

1. The erect **stem**,¹ which may attain a considerable length, branching but rarely.

2. The **leaves**,¹ of relatively small size, and simple form. Their arrangement is in a complicated spiral: at the base of the stem note—

3. A dense mat of **rhizoids** of brownish colour.

At the apex of some specimens will be found merely a bud, composed of young leaves of the vegetative type, other specimens will bear at their apex—

4. Cup-like rosettes of leaves, which assume a bright reddish or orange colour, and protect the **antheridia**; other specimens again may bear at their apex—

5. The mature **sporogonium** or spore-capsule, of which the

¹ Though the terms “stem” and “leaf” are used here, it must be distinctly borne in mind that the members thus named, being parts of the Gametophyte generation, are not strictly comparable with the stem and leaf of Vascular plants, which are parts of the alternate Sporophyte generation.

head or **theca** is supported on a long stalk, or **seta**. Note in specimens which are not too ripe—

a. The **calyptra**, a dry fibrous hood, covering the apex of the sporogonium beneath this is—

b. The lid-like **operculum** with its terminal beak : this lid may be easily detached, disclosing—

c. The pale-grey **epiphragm**, which appears as a transverse membrane, attached at its margin to the capsule by a number of short teeth of the **peristome**.

d. At the base of the theca observe a swelling called the **apophysis**

e. By carefully removing the leaves from the apex of a plant which bears a sporogonium, it may be seen that the base of the seta is enveloped by a closely fitting sheath, the veil or **vaginula**, the origin of which will be explained later.

B.—MICROSCOPIC INVESTIGATION

Gametophyte Generation

II Cut transverse sections of the leafy region of a mature stem of *Polytrichum* : mount some in glycerine, others in chlor-zinc-iodine, or in iodine solution examine them first under a low power, and observe, in those mounted in glycerine, the irregular outline of the section, due to the attachment of the leaves. The mass of tissue composing the section shows two distinct regions :—

1 The outer region, consisting of thick-walled cells, with brownish cell-walls. Light streaks seen in it here and there indicate leaf-traces.

2. A central mass of clearer yellow-walled elements, without contents.

a. Observe under a high power, and examine first this clear tissue (2). Some of the walls are thick and yellow (staining dark brown with chlor-zinc-iodine) ; while others are so thin and colourless, that they can only be seen with difficulty (chlor-zinc-iodine leaves them unstained). This tissue is called **hydrom** : it is specially concerned with the conduction of water,

and represents the xylem physiologically. The largest elements of the hydrom are at the centre, while outwards they become smaller, thinner-walled, and lighter coloured.

b Surrounding the hydrom is a layer of comparatively small cells, with brown walls, living contents, and copious starch. —the **hydrom-sheath**.

c Surrounding this, again, an irregular zone of larger cells, with faint yellow walls, which contain protoplasm, but no starch this is the **leptom**, and may be regarded as representing the phloem. These tissues are best distinguished in sections stained with chlor-zinc-iodine

d The leptom is not clearly limited towards the outside, but passes over into the larger, thick-walled, and brown cells of the **cortex**, which becomes more sclerenchymatous, and deeper coloured towards the periphery of the section its cells contain starch. Note that no intercellular spaces occur between the cells of this or of any other tissue of the Gametophyte.

Observe the presence of leaf-traces travelling through the cortical tissue towards the central vascular strand, which may be taken to represent the stele of the higher plants

At the extreme outer limit is a thin **cuticle**, with small and irregular outgrowths. there is no clearly-defined epidermal layer

III. Cut median longitudinal sections of the stem of *Polytrichum*. mount in chlor-zinc-iodine. Note—

1. That the peripheral **cortical region** consists of elongated prosenchymatous elements, which gradually become parenchymatous inwards. Leaf-traces may be observed passing through to join the central strand.

2. The elements of the **hydrom** are very elongated, and their terminal walls, which are extremely oblique, are very thin, and difficult to see; so oblique are they that in transverse sections they appear to be longitudinal, and form the delicate colourless walls mentioned above, II. *a*. All the walls are smooth and without pits.

3. The elements of the **leptom** are only slightly elongated, with comparatively thin walls, the terminal walls being only slightly oblique: they contain protoplasm and a nucleus, but no

starch, and may be distinguished from the cells of the hydrom-sheath by the dark colour of the walls, and the starchy contents of the latter.

IV. Strip off a few mature leaves . mount one of them in water, with the upper surface uppermost, and observe under a low power that the narrow, linear upper portion is marked on its upper surface by longitudinal striæ (the **lamellæ**), and has a minutely serrated margin the broad, sheathing, basal portion of the leaf, which is closely applied to the stem, is thin and membranous, and is not marked by longitudinal striæ.

V. Cut transverse sections of leaves . this may easily be done by holding the terminal bud of a mature plant between pieces of pith, or by embedding in paraffin, and then cutting transverse sections of the whole bud. Mount all the sections as before, and examine first with a low power . Neglecting the almost circular transverse sections of the stem, recognize—

1. Those transverse sections which have passed through the sheathing basal portions of the leaves : these may be readily distinguished by their broad lateral wings, only one layer of cells in thickness.

2. Those which have been taken from the upper part of the leaf these may be distinguished by their more bulky appearance.

Having recognized these sections, put on a high power and examine them in detail —

1. In the section of the sheathing base of the leaf observe —

- a. The two lateral wings, consisting of a single layer of cells, with thickened outer walls, and but little chlorophyll.

- b. The more bulky central portion, consisting of --

- i. An irregular layer of superficial cells with thickened outer walls, covering both upper and lower surface : beneath these are—

- ii. Bands of **sclerenchyma**, in which the lumen is almost obliterated.

- iii. Within these lies a vascular strand, consisting of elements essentially similar to those composing the central strand of the stem.

2. In the sections of the upper part of the leaf note that the

arrangement of the tissues is for the most part similar to that in the above sections, but rather more bulky, while opposite each of the cells at the upper surface is seen to be attached a series of three to six chlorophyll-containing cells, which represent transverse sections of those longitudinal plates, or **lamellæ**, above observed on the upper surface of the leaf: the uppermost cell in each lamella is enlarged and forked. It is obvious that these chlorophyll-containing lamellæ are separate laterally from one another: they constitute the chief assimilating tissue of the plant.

Sexual Organs.

VI. Take a mature antheridium-bearing axis of *Polytrichum*, and dissect it with needles in a watch-glass, keeping all the detached parts. Examine them carefully with a lens, and observe the following categories of organs—

1. The **perigonal leaves**, which are widened laterally into very broad membranous wings, with a clearly-defined central midrib.

2. The white, club-shaped **antheridia**.

3. The **paraphyses**, which will often be found associated with the antheridia: some of them are simply filamentous, others are more or less clearly spatulate.

VII. Cut median longitudinal sections of a male axis: mount in weak glycerine, and with a low power recognize the several organs above described, and their relative positions: note especially the **antheridia** in the axils of the perigonal leaves. Observe under a high power the structure of a single antheridium: it consists of a short **stalk**, and a club-shaped body, composed of (i) a **wall** a single layer of cells in thickness, and (ii) a central mass of cells of more or less clearly cubical form: these are the **spermatocytes**, or mother-cells of the **spermatoids**.

VIII. Take fresh antheridium-bearing specimens of *Polytrichum*, after some days of **dry weather** (or keep them rather dry for some days, carefully preventing any access of water from above): squeeze one of them **between the finger and thumb**: the antheridia will thus be easily forced from their position, and

may be mounted in water. If they were properly mature, it may then be seen that on contact with water the antheridia burst, and the spermatocytes escape, aggregated in a mass. In each cell of this mass a spiral body may be seen, in active movement ultimately it will escape, owing to mucilaginous swelling of the wall of the mother-cell, as a free **spermatozoid** of spiral form, having two cilia.

IX. Since *Funaria hygrometrica* produces sporogonia at all times of the year, and is very common, while *Polytrichum commune* is reproduced sexually only in the spring and early summer, it will be convenient in most cases to use the former in examining the archegonia. In either case, however, it is a matter of some experience and expenditure of time to get a good series of preparations illustrating the development and structure of the archegonium, and the early stages of the production of the sporogonium.

Take a sod of *Funaria*, with no sporogonia as yet visible upon it, but which bears antheridia—these will be situated at the apices of the shorter axes—many of the longer axes will appear to be terminated by ordinary vegetative leaves, and it is on these axes that the archegonia may be found.

From such buds, after hardening in alcohol, cut median longitudinal sections—if not transparent enough, treat with dilute potash solution, and mount in weak glycerine. Examine first with a low power, when the usual arrangement of axis and leaves may be observed; between the youngest leaves an **archegonium** (or several) may sometimes be detected. If mature, it will be seen to be a flask-shaped organ, seated on a short massive **stalk**: it consists of—

1. An elongated **neck**, more or less contorted, composed of a single layer of cells arranged in four to six rows: these surround a central **canal**, which is filled with mucilage at the time of fertilization, but before maturity there may be seen within it a series of **canal cells**.

2. A lower, enlarged **ventral portion**, consisting of two layers of cells, which constitute the **wall**, and inclose a central space, in which may be seen the naked spherical **ovum**, and above it the smaller **ventral canal cell**.

Sporophyte Generation.*The Sporogonium*

X Having noted the external characters of the sporogonium of *Polytrichum*, as above described (p 201), cut transverse sections of the mature seta mount in glycerine, or glycerine jelly Being a cylindrical organ, the transverse section is circular. Note—

1. The superficial layer of cells, with a definite cuticle and thick yellow walls.

2 A band of brown **sclerenchymatous** cells, which graduate internally into—

3 A thin-walled **parenchyma** with large **intercellular spaces**, and containing chlorophyll.

4 A central strand without intercellular spaces, composed of constituents essentially similar to those in the gametophyte, but on the whole of much simpler structure.

XI. Cut median longitudinal sections of the base of the seta, which is inserted on the apex of the Moss-plant mount as before, and note in the upper part of the seta the superficial layer, brown sclerenchyma, thin-walled parenchyma, and central strand, as above described. Following the seta down to the base, it will be seen that the cuticle and brown sclerenchyma stop short, and are replaced by thin-walled parenchyma with plentiful protoplasm; this tissue of the sporophyte is in close connection with the inner surface of the **vaginula**, which belongs to the gametophyte generation, and originates from the lower portion of the archegonial wall.

XII. Passing now to the apex of the sporogonium of *Polytrichum*, remove the **calyptra**: mount it in water or weak glycerine, and examine under a low power. It consists externally of dry, branched, hypha-like filaments, loosely matted together: the neck of the archegonium may often be recognized at its extreme apex. Open the **calyptra** with needles in a drop of water on a slide, and observe the firm inner cap, which is covered externally by the hairy **indumentum**: the latter is the result of outgrowth of superficial cells of the calyptra, and the

whole originates from the upper portion of the archegonial wall.

XIII. After having noted the external form of the capsule, and of its operculum, transverse and longitudinal sections should be cut. For this purpose it will be well to select young specimens, in which the capsule has not attained more than half its full girth after hardening in alcohol they may be embedded in paraffin, or held in pith, and the sections be cut mount in glycerine or in glycerine jelly.

Taking first transverse sections cut from the middle of the capsule, examine them under a low power, and observe the circular or slightly quadrangular outline of the section; its constituent parts may be recognized as follows:—

1. The **wall** of the capsule, consisting of several layers of chlorophyll-containing cells, of which the outermost has its external wall thickened and cuticularized, like a typical **epidermis**.

2. Internally to this wall is an **air space**, traversed in a radial direction by filaments of cells, containing chlorophyll: these are connected internally with—

3. The **spore-sac**, which is limited internally by—

4. An inner **air-space**: this also is traversed by filaments, which connect it with—

5. The central **columella**.

Examine the spore-sac under a higher power, and, if the sporogonium be of the right age, it will be seen to consist of usually five layers of cells.

- a. Two external layers of thin-walled cells forming the outer wall of the spore-sac.

- b. A central layer of cells with dense protoplasmic contents: this is the **archesporium**, which is originally a single layer, but may undergo divisions as it grows older: ultimately by division of each of the resulting mother-cells into four, and their separation from one another, the **spores** are produced.

- c. The archesporium is sheathed again on the inner side by two internal layers of thin-walled cells, forming the inner wall of the spore-sac.

According to the age of the sporogonium various conditions of the spore-sac may be observed, from the single archesporial layer, to the condition of mature spores.

XIV. Examine median longitudinal sections of a similar young sporogonium, and recognize in it the several parts above noted.

The outline of a median section will show at the base the narrow **seta**: passing upwards to the capsule, it will be found to consist of—

1 The **apophysis**, which appears as a basal swelling limited by a definite epidermis with **stomata**: internally is a mass of lax, chlorophyll-containing tissue.

2. Above this is the real **capsule**, in which will be recognized as before in the transverse section—

a The **wall** of the capsule.

b. The outer **air-space** traversed by filaments.

c The **spore-sac** with structure as before described.

d. The inner **air-space**.

e. The central **columella**.

These severally terminate below in the tissue of the apophysis above they may be traced to a point close below—

3 The **operculum**, which appears as a conical lid, at the top of the capsule.

4 Note carefully a transverse band of tissue of pale, compressed cells, at the base of the operculum this is the **epiphragm** mentioned on p 202, which remains after the operculum is removed. At the margin of it will be seen—

5. The **peristome**, consisting of a series of curved cells, with their walls more or less thickened according to age

In order to see the condition of the epiphragm in the mature capsule, and its connection with the teeth of the peristome, take a ripe specimen from which the operculum has dropped off. With a sharp razor cut transversely through the capsule so as to remove the epiphragm mount in water and examine under a low power. Note that the epiphragm is a thin papery tissue, without intercellular spaces. The **peristome** consists of 64 teeth, connected below with the wall of the capsule, above with the margin of the epiphragm. By drying up of thinner walled cells

between the teeth, spaces are formed, through which the spores can escape. The capsule becomes inverted at the period of ripeness, and the dry spores are dusted out through these spaces.

XV. Scatter spores from the ripe sporogonium of *Polytrichum*, or of some other Moss, over moist soil, and keep them at a moderately high temperature, under a bell-glass, for a few days. The surface of the soil will soon be seen to be overgrown by numerous fine green filaments. Having carefully removed some of these with a needle, and having washed the soil from them, mount them in water, and examine them under a high power. Note—

i. The dark-coloured outer coat of the spore, the **exospore**, which may be found still attached to the filaments after they have attained a considerable length.

ii. The fine filamentous **protonema** resulting from outgrowth of the **endospore**: observe especially the **septa**, which are often oblique; the **branches**, usually arising immediately below a septum; the various development of these branches, either—

a. As relatively thin filaments, with brown cell-walls, and no chlorophyll: these are the **rhizoids**, and they penetrate the soil.

b. As relatively thick filaments, with colourless cell-walls, and containing chlorophyll: these constitute the true **protonema**.

c. As solid buds, which are usually situated at the base of one of the branches such as *a* or *b*: in these solid buds of various ages may be traced the successive stages of development of the **Moss-plant**, which is thus produced as a lateral bud on the protonema.

Cultures of protonema, showing all the most important characters above noted, may be obtained at any time of year by cutting fine sods of *Funaria*, or other Mosses, inverting them under a bell-glass, and growing them in moist air, and at a moderate temperature, for one or two weeks.

It will also be found possible, by culture of detached leaves, and portions of the stem of the Moss-plant on moist soil, and under other favourable conditions, to induce a formation of protonemal filaments by direct outgrowth of cells of those parts.

Observations should also be made on the rhizoids, and protonema of various Moss-plants, by removing them from the soil, and washing them gently with water and mounting in water examination will show the brown underground rhizoids, with oblique septa and no chlorophyll these may rise to the surface of the soil, and develop as a branched, green protonema. or such protonemal filaments may spring from superficial cells of the stem or leaves

In many respects *Polytrichum* is more complex than other Mosses: it is therefore desirable to make observations also on some smaller and simpler example, such as *Funaria hygrometrica*, which is found growing in tufts, on waste ground, walls, &c, especially where ashes are present, *e.g.* where heaps of leaves have been burnt. it is very common, and is easily recognized by its pale colour, and the sinuous form of the seta when in fruit.

In general morphology it resembles *Polytrichum* consisting of a short axis bearing leaves, and attached below by rhizoids to the soil: but the axis is very short, and the leaves pellucid.

Cut transverse sections of the stem of *Funaria*, and mount in weak glycerine, or glycerine jelly it is seen that the stem is of much simpler structure than that of *Polytrichum* at the centre of the section there is a small but distinct strand of thin-walled, small-celled tissue this is the equivalent of the central strand of *Polytrichum*. The rest of the section is made up of parenchymatous cells, whose walls are brownish coloured, and become gradually thicker towards the outside. The peripheral cells usually contain chlorophyll.

Mount a single leaf in water. there are no lamellae present as in *Polytrichum*, but there is a conspicuous midrib, and the rest of the leaf consists of a single layer only of chlorophyll-containing cells.

The sexual organs are as in *Polytrichum*, and are on distinct plants, but not surrounded by any specialized leaves. they are produced at almost any period of the year. The general plan of the sporogonium, and its mode of production, is like that in

Polytrichum: one point of difference is to be noticed, viz, that, when the operculum falls away, no epiphragm is to be seen, but a double fringe of brown contorted **teeth of the peristome**, inserted round the margin of the cavity of the capsule. The tips of the teeth are all free, and move hygroscopically. If longitudinal sections be cut of the young capsules, the structure will be seen to differ from that of *Polytrichum*, (1) in the absence of the inner air-space, the inner wall of the spore-sac being continuous with the columella: (2) the absence of the epiphragm. (3) the greater size and prominence of the peristome. The study of longitudinal and transverse sections of capsules approaching maturity will show that the peristome is composed of an inner and an outer series of teeth, and that the two series are portions of the cell-walls of a single cell-layer which have become separate by the rupture of the thinner parts of the walls.

B.—HEPATICÆ

MARCHANTIA POLYMORPHA, L.

A.—GENERAL EXTERNAL CHARACTERS

1. TAKING a fresh growing sod of *Marchantia*, observe the following external characters with the naked eye, or by help of a pocket lens —

1. The **flattened form**, sinuous margin, and prostrate position of the branched, green thallus.

2. Its dull, dark green upper surface, marked by diamond-shaped areas, and in the middle of each of these a dot, which is a single **stoma**.

3. Projecting from the upper surface there are in most cases small **circular cups**, with a finely crenate margin, in which may be seen numerous dark green flattened bodies, the **gemmæ**: these may be easily detached by slight mechanical disturbance.

4. Note the **organic apex** of the thallus, situated at the base of a terminal depression (compare the prothallus of Ferns) also that the branching is **dichotomous**, though the ultimate development of the originally similar branches is unequal, so that the result is a **sympodium**.

5. In some cases the branches of the thallus may have assumed peculiar forms, together with an **erect position**: these are the branches which bear the sexual organs, and two different types may be easily recognized as borne upon different individual plants, viz.,

a. Branches with a relatively thin stalk, bearing a terminal disk with crenate margin, and having numerous dot-like markings on the upper surface—these are the **male branches**, having the **antheridia** on their upper surface.

b. Branches, also with thin stalks, bearing a terminal star, about $\frac{1}{4}$ inch to $\frac{1}{2}$ inch in diameter: these are the **female branches**, which produce the **archegonia** on their under surface, and ultimately the **sporogonia** and **spores**.

11. Remove a thallus carefully from the soil, and wash with water, taking care not to injure it—examine the organs on its lower surface with a pocket lens, and note especially—

1. The numerous **rhizoids** or root-hairs, attached chiefly to the central midrib.

2. The **amphigastria**, white or purple lamellæ attached to the lower surface of the thallus, and most clearly seen in the regions near the apex, where they are closely aggregated so as to protect the young tissue.

B—MICROSCOPIC OBSERVATIONS

III. Cut transverse sections of the vegetative thallus of *Marchantia*, avoiding at first the cups bearing the gemmæ, and the sexual branches. It is easier to use material hardened in alcohol, and to embed it in paraffin, or to hold it between pieces of pith—but if sections be cut from fresh green material the presence of chlorophyll will be found to be an advantage in distinguishing the tissues. Mount some sections in weak glycerine, others in chloro-zinc-iodine, or in iodine solution: examine under a high power, and, starting from the upper surface, observe—

a. The **superficial layer**, or so-called “epidermis,” consisting of a continuous layer of cells of small size which contain chlorophyll—the continuity of the layer is broken here and there by the so-called “stomata.” these, however, differ from the true stomata of the higher plants in the mode of their development. This layer is attached to the subjacent tissue at intervals only, by continuous vertical plates of cells—the lines of attachment

of these plates to the superficial layer correspond to the limits of the diamond-shaped areas above noted.

b. Beneath the "stomata" are large areas, the **air-cavities**, in which are seen numerous round or oval cells, grouped in simple or branched series, and attached to the lower surface of the cavity: their cell-walls are thin, and consist of cellulose. these cells contain chlorophyll, and are the chief assimilating tissue of the plant.

c. Below this is a massive tissue, which constitutes the great bulk of the section. it consists of oval cells. the walls are thin, and marked with shallow pits. the protoplasmic contents are scanty. in the cells nearer the upper surface there are often numerous starch-grains. Individual cells here and there in this tissue have peculiar mucilaginous, or highly refractive, yellowish or brown, oily contents.

d. Attached at the lower surface of the thallus may be seen organs of two kinds—

1. **Hairs**, or **rhizoids**, which are long and unicellular, and are inserted deeply in the tissues of the thallus. some of these are smooth-walled; others show dotted or peg-like ingrowths of the cell-wall of various form.

- ii. The **amphigastria**, which may now be seen to be plates of tissue one layer of cells in thickness. their cell-walls are often coloured violet or brown.


Returning now to the "**stomata**," note under a high power their structure as seen in a good transverse section. each will appear as consisting of tiers of small cells (four or more in depth), which surround a large central cavity.

IV. Cut tangential sections so as to strip off the so-called "**epidermis**": mount with the outer surface uppermost in weak glycerine: observe under a low power the diamond-shaped areas above described, and a single large "stoma" in the middle of each. Under a high power note—

1. That the cells of the "**epidermis**" contain chlorophyll.
2. That each "stoma" is bounded by four or five of the tiers of cells above described.
- 3. That these cells contain but little chlorophyll.
4. That on focussing downwards it becomes apparent that the

lowest cell of each tier projects into the cavity of the "stoma," so that the channel at that point presents a stellate appearance in surface view

Gemma

V. Remove from one of the cups on the upper surface of the fresh thallus of *Marchantia* some of the **gemmae**: mount them in water, and note under a low power — 

1. The flattened disk-like form of the gemma, with two lateral indentations, and a scar at the base where it was attached to the thallus which produced it.

2. The ordinary chlorophyll-parenchyma of which it is mainly composed.

3. Superficial hyaline cells, from which the rhizoids are subsequently derived.

4. The single cells containing oil-bodies.

It may further be observed that the gemma is in its peripheral part only one layer of cells in thickness, while the central part is a solid mass also that the structure is alike on both sides of the gemma, *i.e.* that it does not as yet show any trace of a dorsiventral character.

VI. Cut transverse sections of a thallus so as to pass through the middle of one of the **cups**: mount in very weak glycerine, or in water, and examine under a low power note—

1. The two lips of the cup, which appear as outgrowths from the upper surface of the thallus, and show more or less clearly the same structure, especially in the lower part.

2. The numerous **gemmae**, in various stages of development, which are attached to the base of the cup by unicellular stalks.

Removing some gemmae from the cups, germinate them on clean moist sand under a bell-glass at a medium temperature, and exposed to moderate light: on examining them after five or six days, they will be seen to be elongated transversely to their original axis of growth, the base of each of the lateral indentations serving as an organic apex: from the lower surface root-hairs have been formed, by simple outgrowth of single cells. After growth has been continued for a longer time the differen

tiation of tissues characteristic of the mature thallus, with "stomata" and air-cavities, becomes apparent on the upper surface, the thallus thus assuming a dorsiventral character.

The Male Branch (Antheridiophore)

VII Cut transverse sections of the stalk of the male branch mount in glycerine, and observe the almost circular outline of the section, with two deep involutions, containing rhizoids, on one side of it this side corresponds to the lower surface of the thallus, from which the male branch is an upward turned continuation.

VIII Cut median vertical sections through the terminal disk mount in weak glycerine, and examine first under a low power observe—

1. The general **outline** of the section, with its flat upper surface.
2. The **amphigastria** and **rhizoids** attached to the lower surface.
3. The **cavities** in the tissue, of two sorts, both opening by narrow mouths on the upper surface—
 - a. **Air-cavities** essentially similar to those of the vegetative thallus.
 - b. **Flask-shaped cavities**, each containing one **antheridium**, which occupies the whole of the cavity each of these also opens by a narrow channel on the upper surface of the disk

Look for a single ripe antheridium which has been cut through longitudinally having found one, examine it in detail under a high power, and observe—

1. The short **stalk** by which it is attached to the base of the cavity.
2. The **wall** of the antheridium, consisting of a single layer of thin-walled cells it is in close opposition to the inner surface of the cavity.
3. The **spermatocytes**, of cubical form, and small size, which together constitute a dense central mass.

The Female Branch (Archegoniophore)

IX Cut transverse sections of the stalk of the female branch mount in glycerine, and note an outline similar to that in the male, with two involutions on one side corresponding to the under surface of the thallus. the other (upper) side shows the chambered structure present in the vegetative thallus, of which it is, again, a specialized branch.

X. Remove the stai-shaped head of a female branch which has attained a considerable length, and examine first the upper surface: with the naked eye or with a lens note the rounded arms, usually nine in number, and the diamond-shaped areas, each having a single central stoma

Turn the head upside down, and observe on its lower surface —

1. The central attachment or **stalk**.
- 2 The radiating **arms**, usually nine in number, which radiate from a central disc
3. The curtain-like pairs of flaps, which alternate in position with the arms the **archegonia** are enveloped by these flaps; and if the branch be an old one—
- 4 The nearly spherical **sporogonia** may be observed protruding from them if these be fully ripe, they may have burst, in which case a yellow flocculent mass may be seen protruding from them, consisting of the **spores** and **elaters**.

XI. Select a female branch which has not yet grown more than a quarter of an inch in height,—cut rather thick transverse sections (*i.e.* in a horizontal plane) through the head of it, mount in glycerine, and examine under a low power: observe—

1. The central **stalk** cut through transversely, and presenting characters similar to those above described.
2. The **arms**, usually nine in number, radiating from the central disc. *
3. The numerous **archegonia**, each of which presents a circular outline from this point of view: they are disposed in

groups alternating in position with the arms, each group being surrounded by the flaps.

Under a high power the following points may be ascertained—

a. That the most mature archegonia are those nearest the periphery, while those of each group are successively younger the nearer they are to the central stalk.

b. That each mature archegonium as seen in section consists of a wall, **one** layer of cells in thickness, which surrounds and incloses a large, naked, nucleated **ovum**.

XII. Cut vertical sections through a female branch of like age to the above. treat as before, and observe in median sections—

1. The **stalk**, on which is borne—
2. The terminal stellate **head**.
3. The **archegonia**, which are flask-shaped bodies suspended in an inverted position—note in a mature archegonium—

a. The long **neck**, consisting of many tiers of cells, which together form a single-layered cylinder surrounding the channel of the neck, which before opening contains a series of **canal cells**. When mature the neck will be seen to be open at its apex, and the canal cells will be no longer visible.

b. The more distended lower ventral part of the archegonium, also surrounded by a single-layered wall, and attached by a short massive stalk to the receptacle.

c. Pay special attention to the contents of the ventral portion: in the mature archegonium the cavity will be seen to be occupied by a single primordial cell—the **ovum**—to which access is gained from without through the channel of the neck: above it a smaller cell may be seen before fertilization—this is the **ventral canal cell**.

XIII. Keep some specimens of *Marchantia*, having mature male receptacles, protected for some days from access of water from above: then place a drop of water on the upper surface of a receptacle, and after a **short** time transfer it to a glass slide, and examine under a high power; there will then be seen

numerous motile **spermatozoids** of elongated, slightly curved form, and they are kept in active motion by two **cilia** attached to the anterior end. In order to make them clearly visible they should be killed, and stained by adding a small quantity of iodine solution, or of osmic acid.

XIV Add a drop of water containing living spermatozoids to a fresh preparation of a female receptacle containing mature archegonia : note the directive influence of the archegonia in attracting the spermatozoids to the neck, which they enter, and are lost to sight in the mucilage which fills it.

MARCHANTIA.—SPOROPHYTE

XV. Cut median longitudinal sections of a female receptacle bearing almost mature sporogonia : mount as before, and note under a low power that the parts of the receptacle remain as before but observe especially—

1. The **perigynium**, a loose sac-like coat, which arises from the receptacle after fertilization, and envelops the archegonium during its further growth.

2. The **wall** of the archegonium, now consisting of two layers of cells, and still bearing at its apex the **neck**, which shows signs of withering.

3. The **sporogonium**, an almost spherical body inclosed by the enlarged venter of the archegonium,—the **calyptra** : the following parts of it are to be recognized—

a. The massive conical foot or **seta** at the end remote from the neck, *i.e.* directed towards the base of the archegonium.

b. The **wall** of the remaining portion of the sporogonium (**capsule**), consisting of a single layer of cells.

c. The hemispherical **sporogenic mass**, with no central columella : in it may be recognized (i.) the **elaters**, long spindle-shaped cells, arranged in a fan-like manner as seen in section ; and (ii.) the **spores**.

Mount a small portion of the yellow flocculent mass which escapes on the rupture of a mature sporogonium, and breathe

gently on it, observing it the while under a low power. note the hygroscopic movements of the spirally thickened elaters, and the consequent separation and scattering of the spores

As *Marchantia* is a highly-specialized type of Liverwort, it is desirable to study also some form in which the vegetative structure is simpler. *Pellia epiphylla* may be taken, a plant which is very common on wet banks, and by streams.

Having noted the flattened form of the pellucid green thallus, and its dichotomous branching, cut longitudinal sections of it if the material be fresh, mount in water; if hardened in alcohol, mount in glycerine, and observe the almost uniform structure of the tissues the chlorophyll is chiefly located at the upper surface. the lowest layer of cells is coloured brown, and gives rise to numerous unicellular rhizoids but there are no amphigastria.

Note especially that the internal cells have their otherwise thin walls strengthened by vertically running, brown bars: where cut through, these are seen to be the result of an equal thickening of the walls on both sides.

Cut also transverse sections, and compare them these demonstrate that the thallus, which is massive in the middle, thins out at the margins to a single layer of cells, that the thickening bars form networks in vertical planes and only occasionally extend in a longitudinal direction. Thus the structure is much simpler than that of *Marchantia*, and both in external form and in internal structure there is a general resemblance to a Fern prothallus.

In *Pellia* the **antheridia** are not borne on special branches, as in *Marchantia*, but are seated directly on the upper surface of the thallus. The antheridia are mature in late spring, or early summer, and will be found in various stages of development in material taken in the early part of the year. They may be seen with the naked eye, as small dots.

Cut median longitudinal sections of a thallus of *Pellia* bearing antheridia: mount as before, and observe the **antheridia** seated singly in flask-shaped depressions of the upper surface,

and roofed over by a circular flap, but leaving a small pore at the centre. As in *Marchantia*, the antheridium consists of a single layer of cells forming the wall, which surrounds a mass of spermatocytes within, and it is attached by a short basal stalk.

The **archegonia** are found in pocket-like cavities at the ends of the female branches: they are arched in by a membranous flap—the **involucre**: a number of them may be found in each pocket. Their structure and development is similar to that in *Marchantia*.

Sporogonia suitable for investigation may be found in plants collected during the winter. Cut median longitudinal sections of the thallus, so as to traverse a sporogonium. In such sections observe—

1. An outer protective flap on either side this is the **involucre**. Within this are the more or less complete remains of the **calyptra**, which is chiefly developed from the wall of the archegonium.

- 2 The **sporogonium**, consisting of—

- a The **seta**, with its enlarged triangular foot, by which the sporogonium is inserted on the tissue of the thallus. the upper part is cylindrical, and undergoes great elongation at maturity

- b. The head or **capsule**, which consists of a wall of several layers. This surrounds a spherical mass of **spores** and **elaters**. The latter resemble those in *Marchantia*. The spores do not remain as simple cells, but divide repeatedly before they are shed.

In spring, when the spores are mature, the seta elongates rapidly, so that the sporogonial head bursts through the calyptra, and is raised a couple of inches or so above the surface of the thallus before the spores are shed. The **dehiscence** is by slits, separating the wall of the capsule into four valves.

THALLOPHYTA

FLORIDEÆ

POLYSIPHONIA FASTIGIATA, Grev.

I THIS seaweed is found on all our coasts, growing in dense reddish-brown tufts, which are fixed firmly on to the thallus of *Ascophyllum* (*Ozothallia*) *nodosum*, Le Jolis. It grows to a length of about two inches, and the thin cylindrical thallus is frequently branched in an apparently dichotomous manner—on some of the plants taken in autumn, roundish bodies are borne laterally (**cystocarps**); on others, irregular yellowish tassels at the ends of the branches—these are the **antheridia**, and they are best seen on specimens taken in early summer; on others again, dark irregularly disposed swellings may be recognized in the substance of the thallus—these are the organs of vegetative reproduction (**tetraspores**).

II The material to be used for microscopic investigation should be either quite fresh, and be kept and mounted in salt water, or better in weak glycerine; or it should be preserved in alcohol.

From material thus treated, select a thallus which does not apparently bear any of the reproductive organs above mentioned: mount a piece of it, including the tips of some of the branches, in 50 per cent. glycerine and water, and observe under a low power—

1 The cylindrical form of the thallus, and the slight inequality of the apparently dichotomous branching.

2. The general structure of the mature parts of the thallus, consisting of—

a. A series of large **central cells**, with dark reddish-brown contents : these are surrounded by—

b. A single layer of **pericentral or cortical cells**, which are arranged with considerable regularity in rings, each ring corresponding to, and surrounding, one of the central cells : the whole thallus is thus built up of successive tiers of cells.

3. Observe also the apices of the branches, which taper off to fine points, each terminating in a single dome-shaped **apical cell**.

Select a good specimen of an apex, and examine it in detail under a high power—observe—

i. The conical ending of the branch, covered by a thick mucilaginous wall, which extends backwards over the more mature parts, and is covered externally by a definite and continuous **cuticle**.

ii. The single dome-shaped **apical cell**, with highly refractive protoplasmic contents, and more or less obvious nucleus.

iii. The successive segments which have been cut off from its base by parallel transverse walls.

iv. The subdivision of the segments by longitudinal walls, so that each segment ultimately forms one of those tiers of cells of which the whole thallus is built up.

In mature parts of the thallus, as also near the young apex, note carefully under a high power the fine protoplasmic strands which extend through the swollen cell-wall, connecting the protoplasmic body of the various cells of the thallus one with another for further study of these recourse must be had to sections of the thallus.

III. Embed, and cut transverse sections of the thallus of *Polysiphonia*, selecting such a part of it as is not too old, *i.e.* about half-way between the apex and the base : mount in glycerine and examine under a high power. It may then be seen in those sections which pass through the middle of one of the tiers of cells above noted that—

1. The section is circular, since the thallus is cylindrical.

2. That it is limited externally by a clearly marked **cuticle**,

and it will be remembered that as *Ascophyllum*, on which it grows, is found about half-tide mark, the plant is exposed to the air for several hours in each tide.

3. The series of **pericentral cells**, of variable number.

4. The single large **central cell**.

5. Note especially the **protoplasmic strands**, which run from the central cell to the several pericentral cells, traversing the cell-wall.

IV. Cut transverse sections of the frond of *Ascophyllum*, so as to pass through the insertion of one of the tufts of *Polysiphonia*—mount as before, and observe under a low power that elongated, thick-walled cells, produced from the base of the *Polysiphonia* plant, penetrate deeply into the thallus of the host, and thus obtain a firm hold upon it, while their own strongly thickened walls will explain further the strength of attachment.

V. Having distinguished with a lens, or under a low power, a specimen bearing tetraspores, mount a portion of it as before directed (taking care that young branches as well as mature ones are represented), and examine under a medium or high power. Note—

1. That the regularity of the tissues of the thallus is disturbed at certain points by dark spherical bodies, which lie embedded in the tissue below the pericentral cells: these are the **tetraspores**.

2. That they decrease in size as the apex of the branchlet is approached.

3. That they have no clearly definite arrangement.

4. That each undergoes a division into four, hence the term “tetraspore.”

5. That they escape by rupture of the layer of pericentral cells: note in older parts the vacant cavities whence tetraspores have escaped.

6. That the mature tetraspores are naked, spherical, and motionless protoplasmic bodies.

VI. Mount as before a small piece of a male plant taken in April, and observe under a medium power—

1. The normal structure of the main thallus.

2. The club-shaped **antheridial branches**, often associated together in groups: each consists of—

- a. A unicellular **pedicel**, by which it is attached to the thallus.
- b. A central linear series of cells, which is almost entirely hidden by—
- c. Numerous, closely aggregated, and small **antheridial cells**.

Mount specimens of antheridial branches from fresh living material in sea-water, and having found an antheridial branch exactly at the period of maturity, observe the partial disorganization of the walls of the antheridial cells, and consequent liberation of their protoplasmic contents, without subdivision, as round non-motile **spermata**.

VII. Having recognized a female plant by observations with a lens on specimens taken in late summer or autumn, mount a portion of it in glycerine, and, examining it under a low power, observe—

1. The normal structure of the thallus, which bears—
2. **Cystocarps** of ovate form: these consist of closely aggregated, small-celled tissue: they occupy the same position as the lateral branches in a vegetative thallus.

3. Compare numerous specimens, and note cystocarps in various stages of development: in those which are mature the carpospores may be seen through the wall of the cystocarp.

VIII. Embed mature cystocarps of *P. fastigiata* in paraffin, and cut from them median longitudinal sections: mount in glycerine, and observe—

1. The short thick stalk of the cystocarp
2. Its wall, consisting of small, closely aggregated cells, and with an opening or **ostiole** at the apex.
3. The central cavity, surrounded by the wall, and filled more or less completely, according to age, with elongated, club-shaped cells, having dark protoplasm, and swollen walls: these are the **carpospores**.

The artificial germination of the spores is a matter of difficulty, but a rough idea of the germination of the spores of red seaweeds may be gained by observing the numerous young plants, of various genera and species, which are to be found attached to the outer surface of almost any one of the larger seaweeds.

PHÆOPHYCEÆ

FUCUS SERRATUS, L. (Wrack)

OBSERVATIONS WITH THE NAKED EYE

1. Of the various species of *Fucus* which are to be found on our shores, the best adapted for laboratory work is *Fucus serratus*. It is to be found near or below mid-tide level, and may be distinguished from other species by its dark olive colour, the flattened form and serrate margin of the branches of the thallus, the absence of swollen "bladders," and the presence of numerous dot-like conceptacles, crowded together on the ends of branches which show no special swelling.

Having recognized the species by these characters, examine a well-developed plant with the naked eye, and note the following parts of the thallus —

1. The flattened **disk**, of irregular outline, by means of which the plant is firmly attached to the substratum.
2. The **stalk**, which in old plants is of compressed cylindrical form, but in young plants it may be clearly seen that it is originally a broad flattened expansion, with a more or less thickened midrib: a comparison of plants of successive ages will demonstrate that the compressed cylindrical stalk results from the thickening of the midrib, and decay of the lateral wings.
3. If this stalk be traced upwards, it will be seen to branch repeatedly, while on following the branches out they gradually assume the flattened form with serrate margin, thus confirming the conclusions which may be drawn from a comparison of

younger plants, that the whole thallus when young was of a flattened form.

The following observations are also to be made :—

A. Examine the **apices** of young, actively-growing branches those branches which bear conceptacles must be carefully avoided, as they do not show such characteristic appearances the extreme apex is emarginate, or depressed, the base of the depression being somewhat flattened, and marked by a slight groove running in the plane of the thallus.

B. Compare a number of apices : in some only a single emargination will be seen, in others two, similar to one another, each having the groove at the base, while others, again, will show an intermediate appearance. from this it may be concluded that the single apical point divides into two of equal strength, each of which may develop into a branch of the thallus similar to the original. thus the **branching is a dichotomy**.

C. On comparison of a number of branches it may be seen that the development of the two branches of a dichotomy is not equal, one being usually stronger than the other this leads to a sympodial development of the dichotomous branch system.

D. Observe the less regular outline of the ends of those branches which bear **conceptacles** : note with a lens the round orifice or **ostiole** in the centre of each conceptacle. When mature, two kinds of conceptacle may be recognized in the species they are borne on different plants they are—

a. Female conceptacles, the contents of which are of a dark olive-green : these contain the **oogonia**.

b. Male conceptacles, with yellow or orange contents : these contain the **antheridia**.

E. If those flattened parts of the thallus be examined which do not bear sexual conceptacles, there will be found, scattered here and there, organs of somewhat similar structure, which contain only barren hairs, and they may be termed **sterile** or **neutral conceptacles**.

MICROSCOPIC EXAMINATION

II. As it is almost impossible to make satisfactory preparations of the tissues of *Fucus* from fresh material, it will be found a great advantage to fix and harden them—the material is to be fixed by treatment with a solution of picric acid in seawater, and after washing, to remove excess of picric acid, it is hardened in successive strengths of alcohol. An alternative method, which gives good results, is to treat the specimens first with 1 per cent. chromic acid, wash with water, and harden in successive strengths of alcohol.

From material thus prepared select a young flattened branch of the thallus in which the midrib is but slightly marked—cut transverse sections from it, mount in pure glycerine, and observe under a low power—

1. The elongated elliptical outline of the section.
2. The more or less enlarged midrib.
3. The grouping of the tissues exposed. recognize especially—
 - a. A compact marginal band of tissue of a yellowish-brown colour, the **cortical band**: this graduates off into—
 - b. The less compact central mass of the **medulla**, consisting of a web of interlacing filaments.

Put on a high power, and examine the tissues in detail. Starting from the periphery, observe that the cortical band (*a*) consists of—

1. A superficial or **limiting layer** of cells, regularly arranged, and elongated radially: the cells are not of uniform depth, and examination will show that they divide by periclinal, as well as by anticlinal walls; in fact, they constitute an active, continuously meristematic layer, and accordingly the term “epidermis” cannot be applied to it in the strict sense. With the exception of the outer wall, which is thick and cuticularized, the walls of these cells are thin, and the protoplasm plentiful, with a nucleus.
2. Immediately below the limiting layer, and separated from it by an irregular line, is a **parenchymatous** tissue, consisting of

cells which appear nearly square in the transverse section. each cell has plentiful protoplasm, a nucleus, and several chlorophyll corpuscles. the walls are more or less thickened, swollen, and stratified. here and there are to be seen **pits** closed by a thin, highly-refractive pit-membrane. These two tissues, (1) and (2), together constitute the **cortical band** (*a*) above recognized under a low power.

(*b*) The above tissue graduates off without definite limit into the **medulla**, of which the chief characteristic is the circular outline of its cells, and the excessive bulk of the swollen cell-walls. here and there filaments will be found running in the plane of section. If sections be stained with Schulze's solution the firmer cell-wall stains pale blue, but the swollen matrix does not stain.

Occasionally a section may be found which has passed through one or more of the sterile or neutral conceptacles. If the conceptacle was young, it would be found to be a still closed cavity of considerable size, filled with mucilage, and with hairs, which originate from single cells of the tissue lining the cavity. in sections of older parts the ostiole would be found widely open, and the hairs much longer.

III. Cut longitudinal sections of a young part of a thallus (*i.e.* close to the apex of a branch), mount in pure glycerine, and examine under a high power: recognize, as in the transverse sections—

a. The **cortical band**, consisting of—

1. The **limiting layer**, which presents similar characters to those seen in transverse sections.

2. The **inner parenchyma**: note that the cells of the inner part of this tissue are arranged in longitudinal rows, having relatively thick, occasionally pitted, lateral walls, and thin transverse septa: this tissue merges gradually into—

b. The **medulla**, which consists of longitudinal rows of cells constituting filaments resembling the hyphæ of Fungi, with numerous thin transverse septa, to which the protoplasmic contents closely adhere.

IV. In order to study the process of thickening of the midrib: cut transverse sections successively of older (*i.e.* lower) parts of

the stalk : treat as before, and compare them. It will then be recognized that, as the thallus grows older, the cells of the limiting layer cease to divide by periclinal, and later also by anticlinal walls : it becomes a quiescent tissue, and is ultimately thrown off ; the inner cortical tissue, however, remains active, the cells increase in size, dividing periclinaly, and form a massive band, easily recognized by the naked eye. The medulla also increases greatly in bulk, many new hyphal filaments being formed, while they differentiate into two series (i) smaller ones, with sparing protoplasm ; (ii) others of larger size, with a granular protoplasmic lining.

V. Sections should be cut through the organ of attachment. Take plants grown on wooden piles, or on limestone rock . in the latter case the lime may be dissolved by dilute hydrochloric acid, and the tissue then hardened in alcohol. Cut vertical sections, and mount as before . note under a low power the irregularity of the surface of attachment, which closely follows that of the substratum, hence the firmness of its hold. Foreign bodies may often be seen embedded in this part of the thallus, and this finds its explanation in the fact that the tissue here consists of hyphæ similar to those of the medulla, and each appears to grow in an independent manner. Examine the section under a high power, and it will be seen that the mass of tissue resolves itself at the surface of attachment into a number of separate filaments, each of which applies itself separately to the surface of the substratum.

VI. Cut thin longitudinal sections through the apex in a plane perpendicular to that of the flattened thallus . treat those sections which are **median** with glacial acetic acid, and mount in a mixture in equal parts of pure glycerine and glacial acetic acid . examine under a medium power, and observe—

1. The outline of the section, showing a depression of the apex corresponding to the groove already recognized with the naked eye.

2. That from their arrangement it may be concluded that the various tissues of the thallus are derived from an initial point at the base of the depression.

3. Examining the tissue at the base of the depression, if the section be median, a single large cell having the form of a

truncated pyramid may be recognized in a central position. **this is one of the initial cells.**

VII. Cut successive transverse sections of the apex, so as to pass immediately below the base of the depression: one of these sections will include **the initial group**, which will then appear to be composed of some four or five cells, of oblong form, placed in a row, side by side.

VIII. Cut transverse sections through the fertile branches of the thallus, so as to traverse the mature conceptacles: mount in glycerine, and examine under a low power. First take sections of the male thallus, and having found a point where a **male conceptacle** has been cut in median section (*i.e.* so as to traverse the ostiole) note—

1. The spherical or flask shaped **cavity**.
2. The **ostiole**, by which the cavity communicates with the exterior.
3. The **hairs**, which almost fill the cavity, and may even protrude through the ostiole
4. The **antheridia**, which are single oval cells, borne often in large numbers on these hairs

It may further be noted that the cavity is lined by a small-celled tissue, from which these hairs arise, and that this graduates imperceptibly into the other tissues of the thallus, which are similar to those of the vegetative parts

IX. Tease out with needles the contents of a male conceptacle in glycerine, mount, and observe under high power note—

1. The thin, colourless, branched hairs, which bear the oval cells (**antheridia**) with their yellowish granular contents.
2. The mode of branching of the hairs which bear the antheridia.
3. Long hairs, branching less frequently, or not at all: these do not bear antheridia.

X. With the above compare sections cut through **female conceptacles**, mounting as before: in its form, and also in its relation to the tissues surrounding it, the female conceptacle is similar to the male; the difference is in the contents, which may be seen to consist of—

1. Barren hairs, which are usually unbranched.

2. **Oogonia**, bodies of relatively large size, and oval form, with a thick transparent wall, and dark granular protoplasm each of these is seated on a unicellular pedicel.

Observe in the largest of the oogonia that the protoplasmic body may be seen to have undergone division into **eight parts** (**ova**), the surfaces of separation being visible as transparent lines.

XI. Observations on the extrusion of the spermatozoids and ova, and on the process of fertilization in *Fucus* must be made with fresh material, and will be most successfully carried out on the coast, the best season for it being winter or spring. Those who have not opportunity for this may succeed in making the observations on fresh material sent from the coast, using a solution of Tidman's sea-salt, 5 ounces to the gallon, in place of fresh sea-water.

If specimens of *Fucus verratus* be kept exposed to the air for some hours (the period of one tide will suffice), an exudation may be observed from the ostioles of some of the conceptacles—on male plants it will be of an orange colour, on female plants of a dark olive-green.

Taking first the male, mount a small quantity of the orange exudation in a drop of fresh sea-water, and examine it under a high power—it will be found to consist of numerous **antheridial cells**, separated from the hairs which bore them—they will be seen to be bursting, and setting free their contents, and the following stages of the process are to be noted—

1. The antheridium is completely closed, the contents are already divided into numerous elongated bodies (said to be sixty-four in number), each having one or sometimes two brightly orange-coloured globules (**chromatophores**): these are the **spermatozoids**, and they may be seen to be in motion before the antheridium bursts.

2. The wall of the antheridium consists of two layers, the outer more firm layer (**extine**) and the inner mucilaginous layer (**intine**): observe the extine to burst at one end, usually at the apex, and the contents inclosed in the intine escape from it.

3. The intine gradually swells, loses its contour, and the

spermatozoids separate, as actively motile bodies of elongated pear-like form. Observe their movements.

To a drop of water containing motile spermatozoids add a little iodine solution, put on a cover-slip, and examine under a high power the two **cilia** may be observed on each spermatozoid attached laterally.

Mount in a drop of fresh sea-water some of the darker-coloured exudation from the female conceptacles, and examine under a high power observe the numerous **oogonia**, with the pedicel often attached note the thick limiting wall, consisting obviously of two layers, an outer (**extine**) more highly refractive, the inner (**intine**) having the characteristic optical appearance of a mucilaginous wall a shallow pit is to be seen on the wall adjoining the pedicel. The contents will be seen in most cases or in all to be divided, as above described, into eight cells—the **ova**. Some of the oogonia will be seen to burst on exposure to the water. watch the process, and note the following stages—

1. A slight convexity appears usually near the apex, the extine having there ruptured, and the intine beginning to protrude.

2. The rupture extends, and the extine gradually shrivels back so as to leave the intine fully exposed, though it usually remains still attached to the extine at the base.

3. The intine swells, and ultimately loses its contour at the apex, and the oospheres, which had meanwhile separated and rounded off, escape into the water as eight naked, non-motile spheres of dark granular protoplasm : in each may be recognized a central clearer area—the **nucleus**.

Into a drop of sea-water in which are free and mature ova, introduce a small number of mature spermatozoids, and watch their movements : they may be seen to approach the ova, to apply themselves closely to their surface, along which they creep : if present in considerable numbers, they give to the ova an irregular rotating movement.

XII. On stones, in districts where *Fucus* abounds, there may be found early in summer olive-brown velvety patches : on examining these with a lens, small club-shaped bodies may be

distinguished, attached by their narrower end to the substratum, and with their broader, free end crowned by a tuft of hairs: these are young plants of *Fucus*, or of one of the allied genera.

Having collected such material, tease it out with needles, in glycerine, and examine the plants thus separated under a low power: the following points are the most worthy of note —

1. The nearly spherical form of the very young plants, which consist of but one, or of relatively few cells, and are limited externally by a definite cell-wall, the formation of this wall is the first obvious change after fertilization

2. The elongated club-shape of the older plants.

3. Their terminal depression, from which hairs protrude.

4. The mode of attachment by means of hypha-like threads of independent growth, similar to those seen in the older plants: some of these threads may be seen quite young, and not yet attached to the substratum.

From such plants as a starting-point, intermediate forms will lead on to the mature *Fucus* plant.

CHARACEÆ

Various members of this family are found growing in stagnant, or slowly flowing fresh water. they are green, but owing to superficial lime incrustations, they may appear white and chalky, especially when dry they are brittle in texture, and are commonly called Stone-Worts. They grow rooted in mud, and put up into the water a branched shoot, which may be a foot or more in length: this bears at intervals whorls of lateral appendages—the “leaves.” In summer and autumn these bear the sexual organs in large numbers, the antheridia being specially prominent owing to their bright red colour. The odour of these plants is characteristic, being like that of onions.

There are two chief genera of the family, viz. *Chara* and *Nitella* (besides other subgenera) the most obvious difference between these is that *Chara* is more robust, the stem and leaves having a peripheral cortex, while *Nitella* has none. A large number of species are distinguished, and as it might be difficult for beginners to recognize any one definite species with certainty from others, the description given below will be confined to the more essential characters of the genus *Chara*, while *Nitella* will be dealt with only incidentally. Fresh material should be used if possible; most of the structural points can, however, be successfully observed on material preserved in alcohol.

1. Examine a mature specimen of *Chara* with the naked eye, or with a pocket lens, and note—

1. The **stem**, which is as thick as coarse packthread, and is marked off into **internodes** of length varying from 1 to 3 or 4 inches: this axis is of unlimited growth, and is terminated by an apical bud.

2. The "**leaves**" or branches of limited growth, which are arranged in whorls, the point of insertion of each whorl being recognized as a **node**: the number in each whorl may vary. Examine the leaves with a lens, and observe that they also show a distinction of nodes and ~~internodes~~ **internodes**; in some species whorls of small unicellular outgrowths (leaflets) may be seen at the nodes: thus the leaves repeat the characters of the axis on a smaller scale.

3. In the axil of one leaf in each whorl is usually found a **branch of unlimited growth**, which repeats all the characters of the main axis.

4. Examine the base of the plant where it is fixed in the mud: very long transparent rhizoids may be seen to be inserted at the nodes.

5. On the inner side of the leaves of plants taken in summer or autumn the **sexual organs** will be found, viz., the **antheridia**, which are globular and of a red colour, and the **oogonia**, which are of a dark olive colour or brown: these sexual organs are seated, in the monœcious species, in pairs, at the nodes of the leaves, and on the inner, adaxial, side of them.

II. Mount a young part of a plant, including at least one whole internode and two nodes, in water: examine under a low power, and observe —

1. The cylindrical **stem**; the internode is covered externally by a small-celled **cortex**, which surrounds one very large **internodal cell**. Note that the cortex is composed of (a) elongated cells, and (b) short cells which project as hemispherical bosses, the whole being disposed in spirally curved rows.

2. The **leaves**, which are also covered in their basal part with cortex, but the cells are straight or only slightly curved. Note that the nodes of the leaves may be seen to be marked by cells which project as round bosses (**leaflets**), or in some species they are elongated. The cortex stops short below the apex of the leaf, which is accordingly terminated by a series of naked cells.

3. Follow the leaves down to their base of insertion at the **node**, and observe a series of short cells which project more or less below the point of insertion: these are the so-called

“stipules.” The axillary bud will also be seen inserted in the axil of the oldest leaf of the whorl.

III. Remove and examine a whole bud under a low power : either an apical or an axillary bud will do. It may be necessary to treat with acetic acid to remove the lime, which is often present in considerable quantity : the bud may be subsequently cleared with potash, and mounted in glycerine. The outer and older leaves will show the characters above noted, but more clearly, since they are younger, and their internodes shorter : the structure of the cortex will thus be better understood in the young than in the mature leaves.

Examine also the cortex covering the short, young internodes of the stem, and recognize the regular arrangement of the cells : the cortex of each internode of the stem is composed of two series of lobes, the one ascending from the lower node, the other descending from the upper : these are in contact at the middle of the internode, and elongate with it as it grows, undergoing a segmentation which is comparable with that of the main axis, and it similarly results in alternating nodes and internodes. Each lobe is thus composed of nodes (3-celled), and unicellular internodes. Compare this arrangement with that of the mature cortex.

Having thus examined the bud, remove the outermost whorls of leaves with needles : then add a little potash and cover the remaining central part of the bud with a cover-slip ; press gently with a needle upon the cover-slip, watching the effect under a low power. The outermost remaining leaves will be pressed aside, and the **apex of the stem** will be exposed.

Observe the terminal dome-shaped, **apical cell**, from which segments are cut off by transverse walls. By comparison of the terminal series of cells from several apices it may be concluded that each segment cut off from the apical cell divides again transversely into two, of which the lower cell without further division develops directly into an internode, the upper divides to form the numerous cells of the node, from which are derived the leaves and the cortex.

Compare this result with the appearance of the leaves when young.

Note that each cell of the apical region contains a single nucleus.

IV. The cells of the Characeæ are well known as good material for showing the movements of protoplasm in the living cell : observations of this are to be made on living specimens mounted in water. *Nitella* may be used, or the naked terminal cells of the leaves of *Chara*. Note that the chlorophyll granules, which lie in the outer band of protoplasm, are stationary the colourless protoplasm below shows however movements by which the granules and clots are carried along, so that a **rotation** takes place round the large central vacuole. If the movements be sluggish, they may be accelerated by gently warming the slide. Note especially the movements in opposite directions on either side of the neutral line, also the relative movements of the contents of adjoining cells.

V. In order to see the rhizoids, which fix the plant at its base, remove one of the lowest nodes of an old plant : wash it gently from mud, &c, and mount in water. numerous long, transparent threads will be seen to spring from the node these are the rhizoids. Observe their smooth wall, and granular protoplasm with central vacuole, and the more or less obvious nucleus. Here and there they branch, the point of branching being marked by an oblique, and peculiarly curved cell-wall from a swelling above the septum the branch-rhizoids spring.

VI. The sexual organs are first to be examined in the mature state. mount a leaf, bearing the bright scarlet **antheridia**, in water, and examine under a low power. Note their **position** at the node and below the oogonium. the spherical **form**, and attachment by a very short stalk. Observe also the surface markings, which indicate that the whole spherical wall is made up of eight unicellular **shields**, of which the four upper are triangular, but the four lower, adjoining the stalk, are four-angled.

Press gently on the cover-slip : the antheridium will burst, and disclose numerous closely packed **antheridial filaments** ; each of these is partitioned transversely into numerous disk-shaped cells, and contains at maturity, a single spiral **spermatozoid** : the form of the latter can be clearly seen under a high power,

and under favourable conditions their escape also as free, spirally coiled bodies, with two cilia.

Attention should be paid to the mode of attachment of the filaments to the shields : in a mature antheridium which has been burst by very gentle pressure, observe that an elongated cell, the **manubrium**, rises from the centre of the inner surface this is terminated by a **head-cell**, which supports six secondary heads, and to each of the latter are attached four of the antheridial filaments : their total number is therefore about two hundred.

VII. Mount in water a leaf bearing one or more mature but unfertilized **oogonia**, or "nucules" as they are sometimes called, and examine under a low power. Observe their **position**, directly above an antheridium then oval **form**, and insertion by a short pedicel. Each consists of an outer coat, composed of five spirally twisted cells, a crown or apical rosette of five cells, and a large central **ovum** : at the base of the latter when young, or after treatment with potash, a short cell is to be seen. Note that a narrow lateral slit may be seen between the cells of the crown at the receptive period, through which the spermatozoids may pass to the ovum. Attempts may be made to see the entry of the spermatozoids by adding a drop of water containing motile spermatozoids to a preparation in water of a mature oogonium.

VIII. Examine mature oogonia, and note the dark colour, and the thickened, lignified wall of the spiral cells, while the oospore itself is surrounded by a thick, colourless wall. Burst one by pressure upon the cover-slip, and it will be seen that the contents consist largely of starch and oil.

IX. The results of germination may be readily observed in *Chara* or *Nitella*, if specimens with mature oospores be kept in water in a bell-glass through the winter. in the spring the oogonia which had settled to the bottom may be found in various stages of germination. Some may be seen still closed at the apex : in others the spiral cells may have ruptured at the apex and two or more transparent filaments project : one of these develops more strongly as the **proembryo**, it divides by transverse walls, and assumes a **green** colour : the other remains colourless and develops into rhizoids. In one of the filaments

which is **far** advanced observe a narrow, disc-shaped cell, two or three cells short of the apex—this is the **stem-node**: from it a pseudo-whorl of leaves arises, and, as a lateral bud, the new *Chara* plant, which at once shows the characteristic alternation of nodes and internodes: observe its position, and successive stages of development.

Lower down is a second node—the **root-node**, which gives rise to rhizoids; these may branch and fix the young plant in the mud.

CONFERVOIDEÆ

ÆDOGONIUM

Various species of plants belonging to this genus are to be found growing in fresh water : they are green, filamentous, unbranched Algæ, and are attached at the base to the external surface of submerged plants, stones, &c. The apex of the filament is in some species extended into a thin, hair-like process : there is often considerable irregularity in the thickness of the filament, by which character, as well as by the peculiar transversely striated markings of the cell-walls, these plants may be distinguished.

1. Mount filaments of *Ædogonium* in fresh water, having gently scraped them off from the surface to which they were attached, and examine them under a low power : observe—

1. The long unbranched filament, of uneven thickness, terminated at the apex either by a rounded cone, or by an attenuated process : note also at the base the **irregularly lobed disc of attachment**.

2. The **septa**, dividing the filament into a series of cells, with green-coloured contents.

3. At the upper ends of many of the cells **transverse striæ** are to be seen : these serve as indications of past cell-divisions.

Examine the filaments under a high power, and pay special attention to these striæ and other irregularities of the otherwise smooth cell-walls. It will then be seen that the striæ are small, sharp-edged, ring-like projections on the outer surface of the wall : also that a single corresponding stria is to be found, more or less distinctly marked, at the base of each cell.

In some cells an **annular ingrowth of the cell-wall** may be

seen immediately below the stria: note its form and connexion with the cell-wall also, when seen in optical section, a central, dark mark: it is here that the ring splits, and by stretching of the ring the well-known process of **intercalation** of a new zone of cell-wall follows. Examine actively-growing filaments, and try to observe various stages of this peculiar process, noting also any indications of the cell-division which follows it, the new septum being formed immediately below the thin-walled intercalated zone.

Passing to the examination of the cell-contents, observe—

1. The colourless **protoplasm**, in which are embedded—
2. The **chromatophores**, which appear as elongated and branched rod-like bodies, more or less closely and irregularly connected together: here and there will be seen highly refractive **pyrenoids** attached to the chromatophores: these are clearly to be distinguished by their dusky purple staining on treatment with iodine solution
3. A single **nucleus**, which is however, difficult to recognize in well-nourished cells.
4. A large central **vacuole**.

II. An examination of fresh filaments may result in the observation of the reproductive organs, and numerous specimens should be looked over with the object of finding them. Thus the reproduction by **zoospores** may be seen, especially in the morning: without the cell having undergone any change of form the cell-wall ruptures by a transverse split, and the protoplasmic body, having previously contracted, escapes through the slit as a motile pear-shaped zoospore, the anterior end of which is surrounded by a fringe of cilia. After a motile period the zoospores attach themselves by the anterior end to some firm body, and, forming a cell-wall, develop into new filaments. Note young plants in early stages of germination: they may be found in numbers attached to submerged plants or stones in waters where *Cedogonium* grows.

III. There is some variety in the details of development of the sexual organs in different species of *Cedogonium*: some species are monœcious, others **diœcious**.

The **oogonia**, or female organs, are most easily observed,

being spherically enlarged cells of the filament, borne singly or several together. in such oogonia note—

i. The rupture of the cell-wall at the period of maturity by a transverse slit.

ii. The beak-like canal, which projects in some species from the slit.

iii. The hyaline receptive spot.

iv. In old oogonia the mature **oospore**, with thick wall, and dense contents.

The **antheridia** are smaller and shorter cells than those of the normal filament. each divides into two cells, the contents of which, without further division, escape, each being a motile yellow **spermatozoid** similar in form to the zoospore, but smaller. Attempts should also be made to observe the germination of the oospore.

SIPHONÆ

VAUCHERIA SESSILIS, *Vauch.*

I. This Alga is to be found growing as a lax green felt on the surface of moist soil (frequently on the soil in pots in green-houses) . it is of so coarse a texture that the separate filaments can readily be recognized with the naked eye, having a somewhat dull-green, glassy appearance. Remove a small portion of this felt: tease it out as gently as possible in water, and examine it under a low power observe—

1. The coarse, green, cylindrical tubes which constitute this Alga.

2 The absence of septa as a rule, though septa may be present occasionally in unhealthy specimens, and are formed in connexion with the reproductive processes.

3 The very irregular monopodial branching

4. Some branches may develop as **rhizoids**, ramifying in the soil, but these are frequently absent altogether.

5. There may be present lateral outgrowths of peculiar form, which are the organs of sexual reproduction (**gametangia**), viz.—

a. Curved cylindrical bodies, which are the **antheridia**.

b Obliquely oval, sessile bodies which are the **oogonia**.

In this species the sexual organs are associated together in groups of two or three, each being inserted separately upon the thallus: a single antheridium is usually associated with one or two oogonia.

II. Put on a high power, and examine the structure of the thallus in detail. Note—

1. The smooth continuous external **cell-wall**: this may be made more apparent by plasmolyzing some filaments with a 2 per cent. solution of common salt.

2. The **protoplasmic membrane**, which lines the wall, and incloses a large central vacuole which runs the whole length of the filament: this membrane may also be more readily distinguished in plasmolyzed specimens. In the membrane are embedded—

3. Numerous oval, or spindle-shaped **chlorophyll corpuscles**: look for some of these undergoing division.

4. Round highly refractive **oil globules**, which are more or less numerous according to the condition of the plant as regards nutrition

Embedded in the protoplasm are also numerous nuclei: but these are small, and only visible after careful staining.

III. On specimens which have been kept under conditions favourable for strong growth, the sexual organs (**gametangia**) are usually to be found in greater or less numbers. Having found a specimen with mature sexual organs, examine first the **antheridium** under a high power.

1. The lower straight portion, or pedicel, rises vertically from the main filament.

2. The curved portion, or **antheridial cell**, differs from the pedicel in the contents being for the most part colourless: it is separated by a septum from the pedicel. When mature, the contained protoplasm forms a large number of small **spermatozooids**, which escape through an opening at the apex.

Next examine a mature **oogonium** in detail: note its sessile position, and the septum which separates it from the main filament: its oblique form and green-coloured, granular contents: when actually mature an obliquely lateral beak is formed, the apex of which becomes gelatinous, so that the motile spermatozooids can gain access to the **ovum**.

IV. Observe the changes which succeed fertilization, resulting in the formation of the ripe **oospore**: the chief are—

1. Formation of a firm wall completely surrounding the fertilized **ovum**, and fitting closely within the wall of the oogonium.

2. An increase in the size and number of the oil globules no cell-division takes place.

V. The reproduction by means of zoospores may readily be observed in specimens grown under favourable conditions in water: a considerable mass of the Alga is to be placed in a porcelain bowl, in water, and exposed in a window after a few days, numerous small plants of *Vaucheria* will be found floating on the surface of the water, or disposed along the submerged surface of the bowl; these have resulted from vegetative reproduction by **zoospores**. In order to see the process, observations must be made in the early morning, or else the culture must be kept in the dark till shortly before the observations are to be made. Shortly after dawn (or soon after the specimens have been exposed to light) some filaments may be seen with a lens to have dark-coloured and slightly swollen ends—these are about to form zoospores—mount some specimens without a cover-slip, taking care to avoid injuring them, and examine under a low power—observe—

1. The swollen end of the filament, with dark, densely aggregated protoplasm, surrounding a vacuole

2. The transverse **septum** dividing the swollen end from the rest of the filament.

If such specimens be kept under observation, the escape of the zoospore may be observed. the following points are to be specially noted—

1. Various changes in the protoplasmic body and vacuole, terminating in the formation of a transparent, and radially striated, outer protoplasmic coat (**ectoplasm**), which lines the cell-wall, while darker, more granular protoplasm (**endoplasm**), including the chlorophyll corpuscles, collects towards the centre.

2. The rupture of the cell-wall by an irregular slit near to the extreme apex of the filament—before the rupture the septum may be seen to present a convex surface to the rest of the filament, indicating greater internal tension in the “zoosporangium”: on rupture, this is relieved, and the septum then projects convexly into the cavity of the empty zoosporangium.

3. The passage of the protoplasmic mass through the opening, by a streaming movement, assisted by more or less marked,

screw-like rotation of the whole body: the protoplasm may undergo division at the time of escape, and two zoospores may thus be formed.

4. The rapid movement of the large zoospore when free, which may be followed with the naked eye, and under the microscope is seen to be rotatory. The motile period lasts but a short time, and varies in different species.

Treat a zoospore, which has just escaped, and is in rapid motion, with a solution of iodine: put on a cover-slip, and examine under a higher power: no cell-wall will be visible, though the solution will in some measure contract the spore. Turning more especially to the ectoplasm, there will be seen numerous **cilia**, arranged in pairs, projecting from the surface of the zoospore, while in the transparent ectoplasm will be seen numerous highly refractive bodies, which stain with iodine: these are **nuclei**, and a careful observation will show that their position is exactly opposite the insertion of the pairs of cilia.

Treat a zoospore, which has come to rest, with a plasmolyzing agent such as 2 per cent. solution of common salt, watching it meanwhile under a high power. the protoplasm will contract, and a fine **cell-wall** will be seen. This result may also be obtained by pressure on the cover-slip.

Mount and examine zoospores which have already come to rest, and by a comparison of them the process of their germination, and the development of new plants from them may be deduced.

CONJUGATÆ

SPIROGYRA

1. In summer, in stagnant or slowly-flowing waters, flocculent freely-floating masses of a vivid green colour, and slimy to the touch, may frequently be found. With the naked eye it may be seen that the masses consist of coiled and tangled unbranched filaments, in which there appears to be no distinction of apex or base.

Mount a few of them in water, and examine them under a low power: note that the simple unbranched filaments are partitioned off by transverse septa into a number of relatively short cells. It will usually be obvious that the filaments are not all alike, and two chief types will frequently be found present—

a Those with two star-like green bodies in each cell. These belong to the genus *Zygnema*, and, as these Algae are not so well fitted for a detailed observation, they may be neglected.

b. Others will be seen to have one or more spirally coiled green bands in each cell. These belong to the genus *Spirogyra*.

A superficial observation of specimens collected at the same time and place will usually show that in different filaments there is considerable variety in size, form of the cells, thickness of the walls, and in the number and arrangement of the spirals. According to these characters (together with those of the zygospore) a large number of species of *Spirogyra* are distinguished. It will be found convenient to select for observation specimens of the largest size, and with the coils of the green spirals furthest apart. Examine such filaments in detail under a high power, in the living state, mounted in water, and observe—

1. That the whole filament is covered externally by a transparent **gelatinous sheath**, with a somewhat irregular outer surface, and showing a radial striation. It is to this layer that the Alga owes its slimy character. It is to be noted, however, that this sheath is almost entirely absent in some species.

2. A firm **cell-wall**, which is more highly refractive. It immediately surrounds the protoplasmic body, and is continuous with the transverse **septa**.

3. The **protoplasmic body**, which consists of—

a. A colourless membrane which lines the cell-wall internally, and surrounds the large central **vacuole**.

b. The green spiral **chromatophores** (one or more) embedded in the protoplasm note their irregular outline, and the numerous highly refractive lenticular bodies (**pyrenoids**) which are contained in them.

c. A bi-convex lens-shaped **nucleus**, suspended in the centre of the vacuole by fine colourless strands of protoplasm, which run to the primordial utricle, attaching themselves to points opposite the pyrenoids.

Stain with an iodine solution, and observe that the colourless protoplasm stains pale yellow, the nucleus a deeper yellow, and it will thus be more clearly seen, as well as one or sometimes two **nucleoli**, which are deeply stained. The pyrenoids stain a dusky purple.

II. The process of cell-division may be very well observed in the filaments of *Spirogyra*. the chief difficulty is however that the process normally takes place at night, beginning about 10 to 12 P.M. If the *Spirogyra* be placed in a flat plate upon a block of ice during the night, and on the following morning the plants be exposed to a higher temperature, the cell-division which had been previously retarded will begin, and the successive stages may be followed.

The following points in the process should be specially noted—

1. The disappearance of the nucleolus.
2. The formation of the striated nuclear spindle and of the equatorial nuclear disc.
3. Division of the chromatin which constitutes the disc, and

the collection of the two halves at the poles of the spindle as the new nuclei, which are still connected by fine threads.

4. The subsequent dilatation of the spindle, the threads becoming more curved, while from it new connecting threads pass to the peripheral protoplasm.

5. Meanwhile granules collect at the equator of the dividing cell.

6. Note also the involution of the chromatophores and their subsequent division.

7. The gradual formation of the septum, beginning at the periphery and proceeding towards the centre.

III In summer or autumn the process of **conjugation** and formation of the zygospores may frequently be observed in *Spirogyra* filaments which are about to conjugate assume a position parallel to one another, and on them the following observations are to be made—

1. Cells opposite one another put out rounded processes, which meet.

2. The wall at the point of junction is absorbed, and thus the canal of communication is formed.

3. Meanwhile the protoplasm of the two cells has rounded off, one (the male) usually doing so earlier than the other (the female)

4. The protoplasm of the male cell passes through the canal, and coalesces with the female to form the **zygote**.

5. The zygote surrounds itself with a thick stratified cell-wall, which is smooth, or shows various markings of the surface according to species.

FUNGI

I. BASIDIOMYCETES

AGARICUS CAMPESTRIS, L. (The Common Mushroom)

I. Examine a brick of "mushroom spawn," such as is sold in the shops for the artificial culture of the Mushroom. It will be found to consist of a compost of dried cows'-dung, loam, and clay, in which numerous very fine microscopic filaments are present, or irregularly branched white bands which may be easily recognized with the naked eye this is the **mycelium**.

Tease out with needles in water some of the mycelium, mount in water, and examine under a low power : note that the white bands recognized with the naked eye are composed of numerous colourless filaments (**mycelial hyphæ**), associated together in a parallel course, while here and there single hyphæ diverge from the rest, and ramify through the compost

II. In order to obtain an actively growing mycelium, bearing "mushrooms," the brick is to be broken into pieces, and these must be buried a few inches deep in a compost of similar nature to that of the bricks : the whole is to be kept moist, at a moderately high temperature, and in the dark. After a period of a few weeks, the compost will be found to be permeated by a mycelium, similar to that in the brick of "spawn," while numerous mushrooms of various sizes will be found connected with it : such a culture as this will suffice for the study of *Agaricus campestris* in the laboratory.

III. Remove a small piece of the mycelium, of an actively

growing culture, mount it in water, and having teased it out carefully with needles, examine it in detail under a high power, and observe—

1. The **hyphæ**, of cylindrical form, and with rounded free ends.
2. The irregular **branching** of the hyphæ.
3. The **septa**, which are transverse, and situated at irregular intervals.
4. Hyphæ may frequently be seen to be incrustated by numerous **rod-like crystals**.

IV. EXAMINE a portion of the mycelium which has begun to produce "mushrooms". with a little care the compost may be entirely removed from considerable tracts of the branched mycelium, and then the relation of the latter to the young mushrooms may be clearly seen. If a series of specimens illustrating the development of the mushroom be examined with the naked eye, the following observations may be made :—

1. That the mushrooms arise from the mycelium itself.
2. That they appear first as irregular rounded, or oval upward growths, of denser texture than the mycelium itself.
3. That on cutting one of the smaller mushrooms longitudinally it appears to the naked eye to be of homogeneous structure.

4. That older mushrooms acquire an enlarged head (the **pileus**), which is supported on a cylindrical stalk (the **stipe**). In this state they are termed "button mushrooms."

5. That as the pileus dilates horizontally the rupture of a veil of tissue (**velum partiale**) about its lower margin exposes a complicated laminated structure formed internally (the **gills**, or **hymenial lamellæ**).

6. Note further the ring or **annulus**, which remains persistent on the stipe of the mature mushroom, and marks the line of rupture of the velum: the corresponding, irregular fringe at the margin of the pileus is also to be recognized.

7. Removing the mature pileus, examine its lower surface, and note the radiating, more or less darkly coloured **lamellæ**, some of which extend the whole way from the margin to the insertion of the stipe, others only a part of that distance.

Lay the pileus of a mature mushroom with its lamellæ downwards on a sheet of white paper for a few hours. on removing it there will be seen on the paper a sort of print of the configuration of the under surface of the pileus, produced by the fall of the minute, dark-coloured **spores**.

V. In order to study the structure of the mushroom by means of sections, it is a great advantage to harden the material, and the following treatment has been found to produce good results. treat the fresh material for about twenty-four hours with 1 to 5 per cent. chromic acid; wash with water, and then successively with 50, 70, and 90 per cent. methylated spirit. the tissues will assume a cartilaginous character, which makes it possible to cut fine sections. in preparing large specimens it is an advantage to cut them up into pieces of moderate size, so that the reagents may gain more ready access to the internal parts.

From material thus treated cut longitudinal sections of the stipe so as to include both peripheral and central tissues. mount in glycerine, and examine first with a low power. observe—

1. The whole is a **spurious tissue**, composed of elongated septate tubes (**hyphæ**), which are closely interwoven.

2. The diameter of the individual hyphæ is less, and they are more closely packed towards the periphery than near the centre of the section.

Examining the sections under a high power it will further be observed—

1. That the hyphæ are branched, while occasionally their endings are to be seen.

2. That they are thin-walled, the transverse or oblique septa being so disposed that the cells are not much longer than broad.

3. The protoplasmic contents of the hyphæ which make up the bulk of the tissue are far from being copious, while no single, well-marked nucleus is to be found in the individual cells.

By careful staining it is possible to demonstrate the presence of numerous very small nuclei.

VI. Cut transverse sections of the stipe, and, treating as

before, observe that the hyphæ appear circular in section, that they are more loosely packed towards the centre than at the periphery, and that, throughout, intercellular spaces are to be recognized.

VII. Passing to the pileus of the mature mushroom, cut tangential vertical sections through it in such a way as to traverse the vertical gills at right angles to their surface: great care must be taken that the surfaces of the gills shall not be injured in the process of preparation, otherwise the basidia and spores which project from their surfaces cannot be observed. Mount in glycerine, and examine under a low power: the chief bulk of the section will consist of the massive tissues of the pileus, which show little or no differentiation; passing downwards to the lower surface where the **gills** or **lamellæ** have been traversed, the sections of these will be seen as fringe-like projections from the lower surface: occasionally branching of the gill may be recognized.

Examine the sections in detail under a high power: the following observations are to be made—

1. The mass of tissue of the pileus consists of a plexus of much-branched hyphæ, with large intervening spaces: it is composed of short cells, similar in their characteristics to those which compose the stipe: the chief difference lies in their arrangement. This spongy tissue becomes denser about the insertion of the lamellæ.

2. The sections through the lamellæ show a differentiation into—

- a.* The central portion (**trama**), in which the septate hyphal filaments are easily recognized running longitudinally down the middle of each lamella, and curving outwards at their ends towards the free surface.

- b.* The **sub-hymenial layer**, composed of shorter, closely-packed cells, constituting a pseudo-parenchyma: this consists of the short-celled, terminal parts of the hyphal filaments which compose the trama.

- c.* The **hymenial layer**, consisting of oblong, closely packed cells, having their longer axes perpendicular to the outer surface: of these cells two types are to be distinguished—

- i. The **paraphyses**, which are somewhat narrower and have smooth rounded ends.
- ii. The **basidia**, which are more bulky, and longer : each bears on its end **two to four fine processes (sterigmata)**; at the extreme tip of each of these there appears a swelling which develops into the mature **spore**. Note various stages of development of the sterigmata, and spores.

VIII Remove a whole gill carefully from a fresh mushroom, mount it on a slide, without any reagent or cover-slip, and examine its surface with a medium power. It may then be seen that the dark colour is due to the dusky spores, which are thickly distributed over the surface of the gill, **two to four being produced from each basidium** : note further the pale colour of the tissue of the hymenium, and the rounded ends of the paraphyses, and of those basidia which are young, or have already produced mature spores.

II ÆCIDIOMYCETES

PUCCINIA GRAMINIS (*Æcidium Berberidis*), Rust of Wheat

A. Puccinia Stage

I. On the stems and leaves of Wheat and others of the Gramineæ in winter, dark oblong patches may often be found, which owe their origin to a Fungus (*Puccinia graminis*) that infests the tissues, and produces the disease called *Rust*

Examine one of these patches with a lens, and note that the superficial tissues of the Wheat are ruptured by a longitudinal slit, and the torn edges are turned back, so as to expose a dense, dark-coloured mass, which protrudes from within the nature of this mass must be studied by means of sections

II. Cut transverse sections of the leaf-sheath, or other diseased part of the Grass plant, taking care that the section shall traverse one or more of the dark patches of Rust mount in glycerine, and examine under a low power. Observe that the structure of the greater part of the section is normal. each dark patch will be seen to be opposite one of the spaces between the vascular bundles, while the epidermis, which normally covers over the tissues, is ruptured. In case it is the leaf which has been cut, dark patches may be observed as rupturing and projecting through the epidermis of both the upper and lower surfaces.

Put on a high power, and in a thin section observe—

1. The thin **hyphæ** of the branched **mycelium** of the parasite (*Puccinia*), which ramify in the softer, succulent tissues, but do not as a rule attack the **sclerenchyma**, or vascular bundles : they may be traced up to the dark patches above noted.

2. The masses of dark brown **teleutospores** or **winter spores**, which are produced by this mycelium, each spore being borne on a thin pedicel · each consists of two cells, with thick walls, differentiated into two layers, the **exospore** and the **endospore**.

III. If pieces of a Grass plant bearing teleutospores be kept in a moist atmosphere (on wet blotting-paper, under a bell glass) in the spring-time, a fine, white, semi-transparent growth will be produced from the teleutospores: this is the **promycelium**. Remove some of these germinated teleutospores carefully with a needle, and mount in water · if this be done without injuring the promycelium, it will be seen under a high power that one or both of the cells of the teleutospore have put out a germinal tube (the **promycelium**) by rupture of the exospore, and protrusion of the endospore · this promycelium divides into four or five cells, each of which (excepting the basal one) produces a conical process (the **sterigma**): the end of each of the sterigmata swells into a small irregularly roundish body (the **sporidium**), which ultimately becomes detached.

B *Æcidium* Stage

IV. Note in early summer on the leaves of *Berberis* irregular bright yellow or red blotches, the tissues of the leaf appearing swollen at those spots, and projecting convexly on the lower surface, while the upper surface of the blotch is usually concave · on the lower surface numerous irregularly distributed yellow cups (**æcidium cups**) may be seen projecting slightly beyond the surface, while on the upper surface also projecting organs of smaller size, and irregular distribution (**spermogonia**) may be seen.

V. Cut transverse sections of a diseased leaf, so as to pass through one of these blotches · mount some in glycerine, others in chlor-zinc-iodine, and examine under a low power: observe

1 That in the thinner **normal** part of the section, between the upper and lower epidermal layers there is a mesophyll consisting of a single palisade layer, and five or six irregular layers of spongy parenchyma.

2. That the greater bulk of the **infected** part is due not so much to increased number of the cells as to the larger size of the individual cells and of the intercellular spaces.

In the sections prepared with chlor-zinc-iodine, if a good staining has been effected, note with a low power that the fungal tissues are but slightly stained yellow, while the tissues of the host are stained in the usual way, chiefly a dark blue. Recognize as the most prominent parts of the parasite—

1. The **æcidia**, cup-like structures, containing a closely packed mass of **spores**, and opening by rupture through the lower epidermis of the host.

2. The **spermogonia**, relatively small, flask-shaped organs opening on the upper surface of the leaf.

Having thus gained a general idea of the sections, examine them in detail under a high power, and note that in the infected patch the cells of the host are apparently embedded in a felt of **mycelium**, consisting of septate and branched **hyphæ**, which traverse and completely choke up the intercellular spaces—they are but slightly stained with chlor-zinc-iodine, while the cell-walls of the host plant assume a dark colour: they are for the most part confined to the intercellular spaces, and especially those round about the æcidia; but it is stated that occasionally they penetrate the cells of the host, and though this is not easy to see, examples of it should be looked for. Turning to the æcidium observe—

1. Its cup-like form.

2. The dense felt of hyphæ at the base of it

3. Immediately above this is the **hymenium**, a layer composed of closely packed, parallel, rod-like cells (**basidia**), arranged perpendicularly to the outer surface of the leaf.

4. The rows of **spores**, which have been successively abstricted from the basidia. observe the hexagonal form, thickened wall, and orange colour of the spores, and the way in which the spores of contiguous rows fit together.

5. The **peridium**, consisting of a single layer of cells enveloping the mass of spores. the form and arrangement of the cells resemble that of the spores themselves, though not so regular: note the thickened and striated outer wall.

Returning to the **spermogonia**, observe—

1. The closely packed, parallel, rod-like hyphæ converging to the centre (**sterigmata**).
2. The minute oval bodies (**spermatia**) abstracted from them, and escaping through the narrow pore on to the outer surface of the leaf.
3. The brush of hyphæ which protrude through the narrow pore.

VI. It is known that the **æcidium**-spores of this fungus will not infect the Barberry plant afresh, but will only germinate so as to infect a Grass plant; thus the fungus is an example of "**Heterœcism**." The spores retain their germinating power only for a short period.

Take some fresh spores from an **æcidium**, and place them in a drop of water on the surface of a fresh leaf of some Gramineous plant after keeping it in moist air for about 48-60 hours, strip off a part of the epidermis, or, better, cut tangential sections of that part on which the spores have been placed—mount in water with the outer surface of the epidermis uppermost, and examine under a medium power—observe that the **æcidium** spores have produced tubular **hyphæ**, which make their way, **through the pores of the stomata**, into the tissues of the Grass plant.

VII. Infect a Grass plant with **æcidium** spores and keep it in a moist atmosphere: in about a week reddish swellings will appear about the points infected, and the epidermis will be ruptured.

Cut transverse sections so as to traverse one of these ruptured spots: mount in water, and observe under a medium power—note—

1. The branched mycelium ramifying in the tissue of the Grass.
2. The ruptured epidermis.
3. The closely packed **uredo-spores** of simple oval form, borne on thin pedicels (basidia). Observe further the **exospore**, rough with small outgrowths: the **endospore**, with four germinal pores, arranged equatorially: note the protoplasmic contents with reddish granules.

The infected Grass plants which have produced uredo-spores should be kept till the autumn, when the patches which before produced uredo-spores only will, on investigation as above directed, be found to bear **telentospores** intermixed with them, and finally to assume the winter condition of containing **telentospores** or **winter-spores** only, in which condition the winter is passed with this stage the study of the Fungus, as above directed, was begun.

III. CLEISTOCARPOUS ASCOMYCETES

EUROTIIUM ASPERGILLUS GLAUCUS

I Keep a slice of dry bread under a bell-glass, until it becomes mouldy. Even a superficial examination of it will show in most cases that more than one kind of Mould is present. Among the rest the most prominent will probably be one which bears roundish, white or pale-green heads closely aggregated, and borne on stalks of about one-sixteenth of an inch in length: this is the conidial form of *Eurotium Aspergillus glaucus*, and the branches bearing the heads are styled the **conidiophores**.

Shake some of these gently with the point of a needle: numerous minute powdery bodies (the **conidia**) will be liberated, and will float away as a fine cloud.

II. From a pure patch of this green Mould remove a small portion with a needle, avoiding mechanical roughness as much as possible: lay it on a slide, moisten with a single drop of alcohol, then add water, and cover gently with a cover-slip. Examine it under a low power, and observe—

1. The stalked **conidiophores**, with large, mop-like heads.
2. Attached to these, the colourless tangled **mycelium** from which they spring.
3. The innumerable detached **conidia** which will be found thickly distributed throughout the preparation.

Having selected one of the largest of the conidiophores, examine it in detail under a high power, noting especially—

1. The robust **stalk**, usually without septa: its wall is clearly defined, and the protoplasmic contents granular and vacuolated.

2. The transversely septate, branched **mycelium**, from which the conidiophores arise as vertically growing branches, usually from a point immediately behind one of the septa : in this as in other cases of branching of the mycelium, the branch grows out at right angles from the hypha which bears it.

3. The swollen spherical **head** of the conidiophore, with its conidia in radiating rows inserted upon it. Examine carefully the way in which the conidia are produced, noting—

a The **sterigmata**, which are peg-like, radiating outgrowths from the head of the conidiophore.

b. The series of **conidia**, in successive stages of development, which have been successively formed by **abstriction** from the sterigmata.

c. The oval form, and spiny surface of the mature conidium.

In order to observe the successive stages of development of the conidiophore, small portions of the Fungus should be taken from the white patches, where the growth is younger, and be treated as before. In these specimens the following points are to be observed—

1. The conidiophore as a club-shaped thick erect hypha

2. The swelling of the head, though it at first remains quite smooth.

3. Minute papillar outgrowths appear on the surface of the head—these are the young sterigmata.

4. The sterigmata elongate, and become attenuated at the tips.

5. The successive stages of abstriction of the conidia from the apices of the sterigmata.

III. In order to trace the germination of the conidia, they should be cultivated under microscopic observation on the slide. For this purpose a moist chamber is to be prepared as directed in Appendix A. It will be necessary to take certain precautions to reduce the probability of access of foreign spores to a minimum, and so insure as nearly as possible a pure culture. Prepare a nutritive solution by boiling French plums in water : this decoction is to be used **very dilute**, and is to be boiled **immediately before starting the culture**, so as to kill any foreign spores which may be already present : with the

same object, the glass slide, cover-slip, and needles are all to be heated in a spirit-lamp, and the porous pad for the moist chamber is to be well boiled in water.

Having made these preparations, place a single drop of the dilute, sterilized decoction on the cover-slip then with a needle, moistened with the sterilized fluid, remove from as pure a tuft of *Eurotium* as can be found a **small number** of conidia, and place them in the single drop on the cover-slip: examine under a low power to see that the number of conidia is small, then quickly invert the cover-slip and place it over the round hole punched in the porous pad. Keep the preparation thus made under a bell-glass, and observe it from time to time under the microscope: if the culture be successful, the successive stages of germination and of further development of the Mould may be watched in detail.

IV. The **perithecia**, and the **ascogonia** (female organs) which give rise to them, are to be sought for on a mycelium which has already produced mature conidia the ripe perithecia (*Eurotium* fruits) may be readily recognized in old cultures on dry bread, as minute yellowish spherical bodies, easily distinguished by the naked eye.

A. Remove a small piece of mycelium which has already borne mature conidiophores, and is thus likely to bear young **ascogonia**, moisten it with alcohol, and then wash off in a watch-glass in water as many of the conidia as possible: tease it out with needles, and, mounting in water, examine under a high power. Observe—

1. That the same mycelium which bears the conidiophores also produces relatively thin whip-like branches, with highly refractive contents.

2. That some of these branches become coiled, at first loosely, but later in a tightly packed spiral of four or five coils, and consisting of several cells, these spirals are the **ascogonia**.

3. That first one, and subsequently several hyphal branches appear below the closely coiled ascogonium, forming an investment round it: the first formed branch is called the **antheridium** (male organ), and comes in close contact with the apex of the coiled ascogonium.

B. From a culture of some six weeks' duration on dry bread pick off with a needle some of the minute spherical perithecia mount them in water, and examine under a low power. observe—

- 1 The round or oval form of the perithecia
- 2 That they are composed of a small-celled pseudo-parenchymatous tissue.
- 3 Then yellow colour.
- 4 Then insertion, each being borne on a single filament of mycelium

The yellow colour is due to an oily substance, which is soluble in alcohol, or in potash solution.

Treat some perithecia with a weak potash solution, mount them in glycerine, and examine under a high power: note—

1. The **wall** of the perithecium, consisting of a single layer of somewhat flattened cells.
2. The cavity surrounded by that wall, filled with bodies of oval form—the **asci**.

In order to be able to examine the asci in detail, mount fresh perithecia in glycerine, press with a needle on the cover-slip so as to burst them, and note—

- 1 The ruptured wall, as before.
2. The oval **asci**, each of which contains eight **ascospores**, of oval shape when young, and bi-convex-lens shaped when mature
3. Other cells may also be found which belong to the pseudo-parenchyma: this is derived by ingrowth from the wall of the perithecium, and is only to be found in young perithecia. at the period of maturity it is completely absorbed.

Among the Moulds which appear with constancy on bread kept under a bell-glass, as also on other organic bodies, is *Penicillium*: it may be readily distinguished from *Aspergillus* by its lower growth, more velvet-like appearance, and blue-green colour, while the latter shows a higher growth, so that the individual conidiophores may be seen with the naked eye, and its colour is an olive-green

Remove a small piece from a pure patch of *Penicillium* which has been recognized by the above characters: tease it out with

needles, then moisten it with alcohol, and mount in water. Examine it under a high power, and observe the branched, septate mycelium, which frequently forms a very dense mat; this is especially the case if it be grown on Pasteur's solution with sugar. Note that certain branches, which grew up from the substratum, end in a brush of closely arranged parallel branches, and that each branch is terminated by a string of conidia—these are formed by basipetal abstriction, in the same way as in *Aspergillus*.

The conidia may be germinated in the same way as those of *Aspergillus*, and with suitable precautions pure cultures may be grown on various nutritive substrata.

IV. PERONOSPOREÆ

PYTHIUM DE BARYANUM

I. Sow seeds of the common garden Cress (*Lepidium sativum*) thickly in a flower-pot cover it over with a glass plate, and keep it well watered, so that the young seedlings grow up in an atmosphere saturated with water. After a few days the head of some of the seedlings may be seen to have bent over, owing to insufficient support of the stem. examination will show that the curvature is a sharp one, so that it is not due to general weakness. further that the stem is thin and flabby at the point of curvature: while fungal filaments may be observed in close contact with the stem at that point, and it is this Fungus (*Pythium de Baryanum*) which is the cause of the disease termed by gardeners "damping off". it is of common occurrence in propagating pits which are kept too warm and moist.

If the Cress cultures be kept damp for some days longer, a thick felt of hyphæ will be formed, which will bind the seedlings together: and finally the disorganization, which usually begins near the base of the hypocotyledonary stem, will spread throughout the seedlings, causing complete rotting

II. Mount part of a stem of one of the collapsed seedlings in water, and examine under a low power observe—

1. That the tissues show an abnormal appearance at the point of curvature, their colour is yellowish, and the individual cells show signs of having lost their turgidity.

2. That numerous colourless branched **hyphæ** extend along the surface of the seedling, being most numerous at the point of curvature, and less frequent further up.

III. Tease out a portion of the infected part, as well as of the healthy part above, with needles in water, and mount so that a part at least of the epidermis shall be seen in external surface view, or sections may be cut, the infected part being held between pieces of pith. in such preparations observe—

1 The healthy part of the epidermis with elongated cells, and occasional stomata

2. The branched, highly refractive, and for the most part non-septate **hyphæ**, running with an irregular, but mostly longitudinal course along the outer surface.

3 Mark especially the points of entry of the Fungus into the host-plant this may be either—

a By **perforation** of the outer wall of a cell of the epidermis and this is by far the more common or—

b. By passage of the hypha **through the pore of a stoma** this is the less common mode.

4 Trace the further course of the hypha through the transparent tissues of the host-plant, noting the **rarity, or complete absence, of septa**.

IV. Place an infected seedling in fresh water, in a flat watch-glass, and examine it at intervals for a day or two under a low power. Many of the filaments will be seen to form swellings at certain points, which assume a spherical form, are filled with granular protoplasm, and are divided off by a septum from the parent filament, while the thin outer wall assumes a darker colour these swollen bodies are the asexual reproductive organs, or **resting spores**: these may be either terminal or intercalary in position on the filament.

It is characteristic of this species that the hypha should be partially or completely emptied of protoplasm for a short distance below the spore.

These spores are capable of withstanding drought, or a temperature below freezing, without losing their vitality.

V. From a culture containing numerous spores, separate a small portion, and expose it in a watch-glass to a relatively considerable bulk of fresh water: examine the culture at intervals under a low power. Some of the spores will be seen to germinate, forming tubular hyphæ similar to those which produced them

VI. Continue at intervals the observation of those cultures which have already produced spores: the formation of the **sexual organs** will frequently be seen to succeed that of the spores.

a. The **oogonium** resembles at first the spore in being spherical, and about of equal size with it, and is partitioned off by a septum: a central spherical mass of protoplasm (the **ovum**) is to be recognized.

b. The **antheridium** arises as a branch, either from the same filament as the oogonium, or from another. its apex is cut off by a septum, and it comes in close contact with the oogonium: a cylindrical process from it passes through the wall of the oogonium, and gains access to the ovum.

c. In more mature specimens the oogonium contains a single round, distinctly walled cell (the **oospore**), which lies freely within it.

Observations may also be made on the Potato Fungus (*Phytophthora infestans*), the mycelium of which permeates the tissues of the Potato plant, while its branched conidiophores project through the stomata.

V. MUCORINEÆ

MUCOR MUCEDO, *Fres*

I. If a slice of bread be soaked in water, and kept under a bell-glass, various Moulds will make their appearance upon it: about the fourth or fifth day there will be seen a Mould, which at first appears white and flocculent, producing long unbranched stalks, which terminate in round heads, white at first, and subsequently becoming black: this will be *Mucor Mucedo*. It may also be obtained on horse-dung kept under a bell-glass, and on various other substrata.

II. Remove a very small piece of the bread bearing the Mould, and tease it out gently in water: mount and examine under a low power: note—

1. Relatively thick, non-septate hyphæ, which ramify in the substance of the bread.

2. Relatively thin branches, which are produced from the thick ones, and themselves, branching repeatedly, produce a very extensive system of minute fibrils.

3. Hyphæ similar to (1), which however grow erect in the air (**sporangiophores**), each bearing at its summit one spherical **sporangium**: this will certainly have been damaged in the process of preparation.

III. Cut off a number of mature sporangia with scissors from the flocculent growth, treating them very gently, so as to avoid damage: mount them in alcohol, and examine them quickly under a low power: observe—

1. The cylindrical **sporangiophores**, each terminated by—

2. The spherical and dark-coloured **sporangium**, with its

dense contents, and its very thin limiting wall, often bearing small radiating projections.

3. Towards the point of attachment to the stalk a clearer space may be recognized in the contents ; this indicates the position of the **columella**.

Add a drop of water, and draw it under the cover-slip with blotting-paper, watching the effect upon the sporangia as the water gains access to the sporangia, they burst suddenly, and the wall may be torn to fragments so minute that it cannot be recognized again. Meanwhile the contents, the swelling of which caused the rupture, gradually distend, and may be recognized as consisting of—

4 Numerous oval **spores**, with smooth walls.

5 An intermediate **muellaginous substance** which is capable of swelling, and thus effects not only the bursting of the sporangium, but also the dispersal of the spores.

6 After the swelling and dispersal of the spores are complete, there will be seen remaining a spheroidal body (the **columella**), which is the distended septum of separation of the sporangium from the sporangiophore. round its base the remains of the wall of the sporangium may often be traced as a ragged fringe.

IV. With similar precautions to those taken in the case of the spores of *Eurotium Aspergillus* (p. 263), sow spores of *Mucor* in a drop of a sterilized decoction of horse-dung, or of French plums, or other suitable solution. the swelling and germination of the spores and the formation of the branched, non-septate mycelium are to be watched, and drawings may with advantage be made at intervals, so as to record the progress of the cultures.

SPORODINIA GRANDIS, Link.

V. *Mucor mucedo* also reproduces itself by means of **zygo-spores**, which are of such size that they may be detected with the naked eye as black bodies which project slightly from the substratum ; but they are **not** of constant occurrence, and may frequently be looked for in vain. Accordingly it will be found

more convenient and successful to study the development and structure of the zygospores in an allied form, in which they are produced in profusion, viz. in *Sporodinia grandis*, Link. (= *Syzygites megalocarpus*, Ehr).

Sporodinia is a fungus which may frequently be found in autumn, growing parasitically on many of the larger, fleshy Hymenomycetes, especially on *Russula*, or *Boletus*: it appears as a greyish or brown flocculent growth, and the zygospores are of such a size that they can readily be seen as reddish-brown bodies with the naked eye. While a part of the mycelium ramifies in the tissue of the host, the zygospores are borne on aerial branches: they may thus be easily recognized.

Tease out a small piece of the flocculent mycelium gently in water: examine under a low power, and observe—

1. The branched **hyphæ**, which are light-coloured, and rarely septate when young, but assume a brown colour, and form numerous transverse septa at irregular intervals as they grow old.

2. The large brown **zygospores**, each supported by two thicker, club-shaped hyphæ (*Syzygites* form)

3. The relatively small **sporangia** borne on branched sporangiophores, and having a structure similar to those of *Mucor* (*Sporodinia* form)

Compare a number of zygospores in various stages of development, and observe in them the following points:—

1. The swelling of two neighbouring mycelial filaments (**suspensors**), and their assumption of a position with their two swollen ends opposite one another.

2. The formation of transverse **septa** cutting off the apical part of each suspensor, thus forming the two **gametes**.

3. The two **gametes** in close contact with one another, while the walls at the point of contact are gradually absorbed, the absorption beginning at the central point: the two protoplasmic bodies thus coalesce to form the **zygote**, or **zygospore**.

4. The increase in size of the zygospore, its contents becoming dense and oily, while the wall at the period of maturity consists of the following successive layers—

- a. The **primary membrane** of the gametes, which remains thin, but persistent as an external covering.

b. The **epispore**, which is a dark-coloured firm or brittle layer, with hemispherical wart-like outgrowths from the surface.

c. The **endospore**, which is thicker and more transparent.

Note how numerous though irregular are the septa in the mycelium which has produced zygospores.

It is not an uncommon thing in *Sporodinia* to find that the two gametes may not come in contact, and no zygote be found, but still each gamete may develop into a body resembling a zygospore in the character of the wall, the contents, and in the mode of germination. These bodies are called **azygospores**.

APPENDIX A

THE following list of reagents is not intended to be an exhaustive catalogue of the various substances in use in the Botanical Laboratory—it includes, however, those reagents which are considered to be of the greatest importance in elementary teaching, together with notes on their proper preparation, and uses.

Acetate of Potash. A strong solution in water is used as a mounting medium for preparations of green parts of plants—in this solution they retain their green colour for a long time. Aluminium acetate may also be used for the same purpose.

Acetic Acid. This is usually used as a dilute solution in water (1 per cent)—it dissolves calcium carbonate with evolution of bubbles of CO_2 . It brings out the nuclei very clearly, and with this object in view it is used with methyl-green—it may also be employed as a corrective after treatment of a preparation with potash, if the tissues have become too transparent. Glacial acetic acid is also sometimes used in the preparation of the apex of *Fucus*.

Alcohol is of universal use as a solvent, precipitant, and hardening agent. Absolute alcohol is the best, but for most ordinary work strong methylated spirit will do. It dissolves chlorophyll and other colouring matters, resins, ethereal oils, and some fixed oils: wax is soluble in hot alcohol. It precipitates some substances, such as sugars, inulin, and asparagin. It coagulates proteids, and has a peculiar action on some crystalloids. It acts as a hardening agent on cell-walls, sometimes rendering them too brittle: this may be overcome by soaking

the material, before cutting sections, in a mixture of equal parts of alcohol and glycerine.

Alkanna (the root of *Anchusa tinctoria*) is used as a test for resin, caoutchouc, and oils. The alcoholic solution of alkannin, as supplied by the dealers, may be used for this purpose ; but it is found better to use sections of the dry alkanna root.

Ammonia. The solution in water is often used for clearing preparations instead of potash, as its action is less intense. It is used with nitric acid as a test for proteids, and with copper sulphate as a solvent for some forms of cellulose (see below, Copper Sulphate).

Ammonium Molybdate, used, dissolved in a strong solution of ammonium chloride, as a reagent for the detection of tannin, with which it gives a voluminous yellow precipitate.

Aniline Sulphate and **Chloride** are used as reagents for lignified cell-walls, which they stain yellow, while no other parts of the tissue are coloured by them. A saturated solution of either of these substances is made in distilled water, filtered, and a few drops respectively of sulphuric or hydrochloric acid are added, so that the solution shall give a distinctly acid reaction : or a solution may be made in alcohol, and then be diluted with water.

Asphalte is used for sealing up slides in which glycerine has been used as a mounting medium : it is liable to become very brittle after a time, and, to prevent the cement breaking away, it may with advantage be covered with a layer of gold-size. It may be obtained ready for use from the dealers.

Benzol, used as a solvent for various substances, e.g. the coagulum of latex, ceric acid, &c.

Brunswick Black may be bought ready prepared from dealers in microscopic requisites : it is used for sealing up slides.

Callus-reagent of Russow is prepared by mixing equal volumes of chlor-zinc-iodine, and of the solution of iodine in potassium iodide : it stains the callus of sieve-tubes a deep brown.

Canada Balsam is to be used dissolved in benzol and in such proportion that it shall have the consistency of syrup. It is used as a mounting medium for sections previously treated

with alcohol, and then, with either oil of cloves, turpentine and creosote, or cajeput oil ; it is also used for sealing up slides

Cane-Sugar. The concentrated solution in water is sometimes used, together with strong sulphuric acid, as a test for proteids. A dilute solution (1 per cent, or more) is useful for mounting living cells for observation under the microscope.

Carbolic Acid (see Phenol).

Carmine. The two best preparations of carmine are those of Beale and Thiersch.

1. Beale's Carmine — To prepare this, 0.6 gramme of carmine is dissolved in 2 c.c. of boiling solution of ammonia ; the solution must then stand for an hour or so to cool, and to allow of the escape of the superfluous ammonia ; to the solution are added 60 c.c. of distilled water, 60 grammes of glycerine, and 15 grammes of absolute alcohol. The mixture must be allowed to stand for some time ; it is then to be filtered.

2. Thiersch's Carmine — 4 grammes of borax are dissolved in 56 c.c. of distilled water, to this 1 gramme of carmine is added, and then twice its volume of absolute alcohol is added to the liquid. After filtration the liquid is ready for use.

Carmine has but little differentiating power — it readily stains the protoplasm and the nucleus ; Thiersch's preparation is especially useful for bringing out the structure of the nucleus. It can very well be used for sections which have been previously treated with picric, chromic, and osmic acids. The time during which the section is to be exposed to its action varies very much — the rule is that the most satisfactory results are obtained by a prolonged immersion in a dilute solution. In case of over-staining, the sections may be washed for a moment in water to which a trace of ammonia has been added.

Preparations stained with carmine are best mounted in glycerine. (See also Picro-carmine.)

Chloral Hydrate is used, together with iodine, for the detection of starch-grains included in the chlorophyll-corpuscles. Dissolve 8 parts chloral hydrate in 5 parts of water, and add crystals of iodine, which will dissolve slowly and colour the solution. The material to be tested should be bleached with alcohol, and then be laid in the solution for twelve to twenty-four hours.

Chloroform is used as a solvent for various substances, *e.g.* oils, coagulum of latex, &c.

Chlor-Zinc-Iodine (Schulze's Solution) is the best differentiating reagent, and the one most generally used, but the chief objection to it is that, as in the case of other preparations of iodine, the stain is not permanent. There are various ways of preparing it, but the best is as follows —

1. Dissolve 110 grammes of zinc in 300 c.c. of pure hydrochloric acid, and evaporate to 150 c.c. (sp. gr. 1.8)

2. Dissolve 12 grammes of potassium iodide in as little water as possible, and add 0.15 grammes of crystals of iodine

3. Mix (1) and (2).

This reagent may however be obtained ready prepared from dealers in microscopic requisites. It may be used either for fresh material, or after treatment with picric acid, or alcohol. The colouring of cellulose walls is intensified if the objects have been previously treated with potash, and the alkali thoroughly washed out.

Under this reagent cellulose walls turn blue, or violet, lignified walls yellow, or various shades to a sherry brown, corky walls yellow or brown, protoplasm brown, while starch-grains swell and turn blue.

Chromic Acid. A strong aqueous solution of this acid, 10 per cent., dissolves lignified and cellulose cell-walls²; cuticularized cell-walls resist its action; but they become very transparent, and may be easily overlooked. A dilute solution brings out the stratification of cell-walls very clearly. A 1 per cent. solution may be used in the preparation of Seaweeds.

Clove-oil is used as a clearing agent before mounting specimens which have been treated with alcohol in Canada balsam.

Copper Sulphate is used in the preparation of Fehling's fluid (see below), and the preparation of ammoniacal solution of cupric hydrate (see below).

Corallin (Rosolic Acid). A solution of corallin in a 30 per cent. solution of sodium carbonate colours lignified tissue, the callus of sieve-tubes, and starch-grains pink.

Creosote is used together with turpentine as a clearing agent before mounting in Canada balsam: 1 part of creosote and 4

parts of turpentine are to be shaken well together, and set aside till the cloudiness formed on their first mixing disappears

Cupric Hydrate. The ammoniacal solution of cupric hydrate is used as a solvent for pure cellulose. To a solution of copper sulphate in water add dilute potash collect the precipitate on a filter, wash with water, and then dissolve it in a little strong ammonia this solution, which is of a dark blue colour, must be prepared fresh each time it is required for use

Dammar, dissolved in warm turpentine, and evaporated to the consistency of syrup, is sometimes used as a mounting medium instead of Canada balsam it does not set so firmly as balsam, and it is well to seal up slides in which it has been used.

Ether is used as a solvent for wax, oils, &c. When very small objects have been embedded in paraffin or cocoa-butter, it may be convenient to dissolve off the fragments of embedding material with ether the small sections will then be readily found, and collected.

"Eau de Javelle" is recommended as a clearing agent for growing points, and other merismatic tissues the cell-contents swell under its action, and the cell-walls which remain may then be easily seen It is prepared by adding to 2 pints of water 2 ounces of chloride of lime, and 4 ounces of carbonate of potash or of soda Objects treated with it are to be washed with water, then with dilute acetic acid, and should be mounted in glycerine.

Eosin is used in strong solution in alcohol, or in water, for demonstrating the structure of sieve-tubes

Fehling's Fluid is used as a test for grape-sugar the following directions for its preparation are given in Foster's *Practical Physiology* -

a. Dissolve 34.65 grammes of pure crystallized cupric sulphate in about 160 c.c. of distilled water.

b. Dissolve also 173 grammes of pure crystallized potassic-sodic tartrate in 600 to 700 grammes of sodic hydrate (sp. gr. 1.12).

Add (a) to (b), stirring well to cause a thorough mixture, and dilute with distilled water to 2 litre.

Fehling's fluid should be fresh made whenever it is required,

since it decomposes on keeping : it will keep some little time if kept in a cool place in the dark, and in completely filled, well closed bottles (Hoppe-Seyler).

The solution (*b*) may be prepared, and kept for adding to (*a*) freshly prepared when required

Before using a kept solution to test for sugar, always boil a little of it by itself to see if any reduction will take place.

From 1 c.c. of this solution the copper is completely reduced by 0.005 grammes of grape-sugar

Ferrous Sulphate is used in dilute solution in water, to which a drop of nitric acid has been added, as a test for tannin

Fuchsin is used in solution in alcohol, for bringing out the structure of thickened cell-walls, and especially the outer walls of the epidermis, and corky walls. the sections should have been previously treated with alcohol. When a section has been stained with fuchsin, and washed in absolute alcohol, the coloration is removed from all parts excepting the corky and cuticularized walls

Glycerine is the most generally used medium for mounting, as it has the advantages of a high refractive index, and of not being subject to evaporation. It may be applied either pure or diluted. pure glycerine is to be used, after hardening in alcohol, when it is desired to observe the details of the protoplasm, e.g. in the preparation of the contents of the embryo-sac ; dilute glycerine (1 part glycerine, 1 part water) is, however, of most general use.

Glycerine Jelly is a suitable mounting medium for many objects : it may be bought ready for use from dealers in microscopic requisites ; or it may be prepared according to Kaiser's receipt, as follows. — 1 part by weight of finest French gelatin is to be soaked for about two hours in 6 parts of distilled water ; 7 parts of chemically pure glycerine are added, and to about 100 grammes of this mixture 1 gramme of carbolic acid is added. The whole mixture is to be warmed and continually stirred for 10-15 minutes, till the fluid is clear, and then to be filtered through glass-wool.

Gold Chloride is sometimes used in a 1.0 per cent. or 0.5 per cent. solution in water as a delicate stain for protoplasm.

Gold-Size is to be obtained from dealers in microscopic requisities. It is used for sealing up slides, and a layer of it may with advantage be applied after sealing with asphalte, or Brunswick black.

Gum Arabic is occasionally used as an embedding medium for very small objects.

Hæmatoxylin A number of preparations of this colouring-matter are in use, of these the following are those generally employed for vegetable tissues.—

1. Alum Solution of Hæmatoxylin.—Dissolve 0.35 gramme of hæmatoxylin in 10 c.c. of water, and add to it a few drops of a solution of alum consisting of 1 gramme of alum to 10 c.c. of water.

2. Kleinenberg's Hæmatoxylin.—Saturate some 70 per cent. alcohol with calcium chloride, let the mixture stand for twelve to twenty-four hours over alum, shaking occasionally, add 8 parts of 70 per cent. alcohol, filter, and then add a solution of hæmatoxylin in absolute alcohol until the liquid has a purple-blue colour; let it stand in a corked bottle exposed to sunlight for about a month; it is then fit for use. The liquid is to be diluted as required with alum solution. This preparation is most generally employed, and it may be bought from the dealers ready for use.

3. Expose a few crystals of hæmatoxylin to the action of gaseous ammonia in a watch-glass under a bell-jar, then add water, and a good colouring fluid is obtained. The disadvantage of this is that it has to be freshly prepared every time it is required.

The alum solutions will stain all parts of the cell, including the cell-wall. Their special uses are (*a*) to make the cell-walls more evident when they are naturally transparent and colourless; (*b*) to stain the protoplasm, so as to make its intimate structure apparent; (*c*) to stain the nucleus, so as to demonstrate its presence and to show up its structure.

The ammoniacal solution is especially adapted for differentiated staining. If a dilute solution be used, the first thing to become stained is the chromatin of the nucleus, then, after a time, the rest of the nucleus (achromatin), then the protoplasm. The cell-walls do not stain with this fluid, or only slightly.

Kleinenberg's hæmatoxylin stains in a few minutes, whereas the alum solution is much slower in its action.

Hæmatoxylin may be used either for fresh material, or for sections which have been previously hardened with alcohol, or with picric or chromic acid. In the latter case the sections must be washed repeatedly in distilled water to remove every trace of the acid, which, if present, would interfere with the proper action of the hæmatoxylin. If the section becomes too deeply stained, as sometimes happens when the alum-hæmatoxylin is used, the excess of colouring-matter may be removed by washing with a solution of alum in water.

Sections stained with alum, or with Kleinenberg's hæmatoxylin, are to be mounted in Canada balsam, or Dammar; those stained with the ammoniacal solution are to be mounted in glycerine.

4. Haidenham's hæmatoxylin is specially useful for microtome sections fixed upon the slide. Two solutions are needed —

A. Solution of iron alum

B. Solution of hæmatoxylin

These can be obtained ready prepared from dealers.

The sections should be soaked out in water, and placed in solution A for five to ten minutes; wash rapidly in water, and immerse in solution B for one-half to one hour, or longer. On removal, rinse again with water, and immerse in solution A, watching carefully the process of destaining, which can be stopped at the right moment by washing in water.

The sections can now be counter-stained, for instance with Bismarck brown dissolved in alcohol, and mounted in Canada balsam. The result is that the chromatin of the nuclei is stained more or less deeply according to the degree to which the staining has been carried, while the cell-walls will be indicated by the Bismarck brown.

This method is especially useful for meristematic tissues.

5. For anatomical detail of mature tissues the following double stain is recommended —

Reagents : (1) Kleinenberg's hæmatoxylin, ordinary solution, diluted one-half with alcohol; (2) strong solution of safranin in half-and-half alcohol and water.

Sections of the material which had been hardened in spirit are to be placed in the safranin solution in a watch-glass for half an hour or longer, as required—remove to hæmatoxylin solution for two to five minutes: wash in water with a little alcohol in it, taking some of the hæmatoxylin over with the section: after a few minutes treat with absolute alcohol, then oil of cloves, and mount in Canada balsam.

Hoffmann's Blue. Used in solution in dilute alcohol slightly acidified with acetic acid—it is a useful reagent, inasmuch as it stains the protoplasmic cell-contents and not the cell-wall—it stains also the callus which closes the perforations of the sieve-plates during the winter in perennial plants. It is also used, together with sulphuric acid, for demonstrating the continuity of protoplasm through cell-walls—in order to do this a small quantity of the dry substance is dissolved in strong sulphuric acid in a watch glass—sections, preferably of fresh material, are immersed in it for a short time, then washed with water, and mounted in glycerine.

Hydrochloric Acid. Used, in very small quantity so as to give an acid reaction, with aniline chloride, phloroglucin, or carboic acid, as a test for lignin. By itself the acid turns lignified cell-walls yellow; when its action is prolonged, the cell-walls become violet, owing to the presence of various substances, such as phloroglucin, coniferrin, and pyrocatechin.

Iodine is one of the most useful reagents—it is prepared in various ways. The most important are the following—

i. Make a strong solution of potassium iodide in distilled water, add to this crystals of iodine and set it aside for some hours, shaking it occasionally—dilute this solution with distilled water to the colour of brown sherry. The reagent may also be prepared by diluting the *liquor iodi* of the Pharmacopœia. This is the ordinary iodine solution in common use in the laboratory.

ii. The alcoholic solution may be prepared by dissolving crystals of iodine in alcohol, and diluting with alcohol to a dark sherry colour; also by diluting the *tinctura iodi* of the Pharmacopœia: in the absence of water this solution does not give the blue reaction with starch.

iii. A solution of potassium iodide and iodine in pure glycerine is sometimes used in the treatment of crystalloids.

iv. The solution of iodine in chloral hydrate is used for detection of included starch-grains (see above, Chloral Hydrate).

v. For the solution in chloride of zinc (Schulze's solution), see above, Chlor-Zinc-Iodine.

The ordinary solution of iodine (1) stains proteid substances, and especially the nucleus, brown; cellulose faintly yellow; cuticularized and lignified walls yellow; gum purple and starch blue. Together with sulphuric acid, iodine colours cellulose blue, a reaction similar to that with chlor-zinc-iodine.

Methylene Blue is used in solution in water—it stains the cell-wall, but not the protoplasm.

Methyl-green. A tolerably strong alcoholic solution of this is used. The sections of the object, which must have been previously kept in absolute alcohol, are to be treated with the staining-fluid for from 5-25 minutes, then quickly washed with distilled water, and mounted in glycerine. The nucleus stains of a green or bluish-green colour, the protoplasm remaining uncoloured. It is especially good for staining nuclei which are dividing, and for bringing out the nuclei in the cells of Fungi, and of the Siphonæa, for which purpose Strasburger recommends the following method—The fresh object or section is mounted in 2 per cent. acetic acid, to which a little methyl-green has been previously added—the nuclei are fixed almost instantaneously and at the same time stained. These preparations may then be washed in 1 per cent. acetic acid, and be mounted in weak glycerine and acetic acid. Objects stained with methyl-green fade very rapidly.

Methyl-violet. This is used in concentrated alcoholic solution. It is especially useful for staining Bacteria. A few drops of the solution are added to 15-20 c.c. of distilled water, and a drop or two of the mixture should then be placed on the Bacteria-membrane (zooglæa), and be allowed to remain there for a short time until the membrane appears to be coloured; if the solution used be too strong, the substance between the Bacteria will become stained. The colouring matter is then washed off

with distilled water, or better with a 10 per cent solution of acetate of potash. The preparation may then either be allowed to dry in the air and then be mounted in Canada balsam, or it may be mounted in a 50 per cent. solution of potassium acetate in water.

A useful preparation of methyl-violet is the following — Some of that substance is dissolved in strong sulphuric acid, forming a brownish-green solution on the gradual addition of water the violet colour reappears. This is especially useful for sieve-tubes. If a section be treated with this fluid for a short time, and be then washed with water, it will be found that the cell-walls have become swollen and transparent, that the protoplasm has become deeply stained, and that the sieve-plates are very well brought out. Lignified tissues treated with this fluid assume a yellow colour, as they do when treated with aniline sulphate.

Moist Chamber (see Water).

Nitric Acid colours cuticularized cell-walls and proteids yellow; it also causes swelling up of cellulose and of lignified cell-walls. When diluted with water it is useful for dissolving the crystals of calcium oxalate which are frequently present in the cells. It is used with ammonia as a test for proteids (xanthoproteic reaction), with potassium chlorate as a test for suberin, and as Schulze's macerating fluid.

Olive Oil is used as a medium for mounting aleurone-grains, so as to see them unaltered.

Orcin. A solution in alcohol is used as a test for inulin. Sections are to be soaked in the solution and subsequently warmed with strong hydrochloric acid: an orange-red colour shows the presence of inulin.

Osmic Acid is used in 0·1-1·0 per cent. solution in water, for fixing and hardening protoplasm: it also stains fats black. The solution should be kept in a well-stoppered bottle in the dark.

Paraffin is used as an embedding medium for small or delicate objects. Paraffins of varying hardness and temperature of melting-point may be obtained: the best for ordinary use is a mixture which shall melt at a temperature of 50° to 60° C.

• **Phenol (Carbolic Acid).** Used, together with hydrochloric

acid, as a test for lignin. The best preparation of it is its solution in hydrochloric acid ; this is prepared by dissolving carbolic acid in warm hydrochloric acid, adding, whilst the mixture is cooling, sufficient hydrochloric acid to dissolve any precipitate that may be formed. Lignified cells, treated with this mixture and exposed to sunlight, assume a bright green colour in consequence of the presence of coniferin. It may also be used, instead of creosote, together with turpentine, as a clearing agent, before mounting in Canada balsam. A small quantity is to be added to glycerine jelly to prevent the growth of Fungi.

Phloroglucin. Dissolve some phloroglucin in methylated spirit, and gradually add strong hydrochloric acid till precipitation begins, the liquid is then ready for use : in sections treated with it lignified walls assume a bright red colour.

Picric Acid. A saturated solution in water is very generally used for fixing the protoplasm of the cell as nearly as possible in the form which it held during life. It is, however, objectionable, owing to the difficulty in completely washing it out from the specimens before hardening in alcohol, and in most cases treatment at once with absolute alcohol is to be preferred. In some cases, such as delicate Algae, it is well to dilute the saturated solution with an equal volume of water.

Picro-carmin (or ammonium picro-carminate) is prepared by adding a strong ammoniacal solution of carmine to a quantity of concentrated solution of picric acid in water, until a precipitate begins to be formed ; it is then evaporated to about one-fifth of its bulk, filtered, and the filtrate is evaporated to dryness. The crystalline residue is dissolved in water so as to make a 5 per cent. solution, and this may be diluted as occasion requires.

Another method (Gage) is to dissolve a quantity of picric acid in 100 parts of water, and an equal quantity of carmine in 50 parts of solution of ammonia ; these are then mixed, filtered, evaporated to dryness, and the residue dissolved in 100 parts of water.

Picro-carmin is used especially for staining nuclei, the staining being more uniform than when carmine alone is used ; it has this further advantage, that a prolonged exposure to it does not produce overstaining, as is the case with the other

preparations of carmine. The objects should be previously kept for some time in absolute alcohol. If it be desired to retain the double staining which this reagent produces, the sections must be mounted at once in glycerine, but if the carmine staining only is required, the sections must be washed in water, which will dissolve out the picric acid. When stained sections are mounted in glycerine, a small quantity of picro-carmine must be added to the glycerine in order to preserve the colours.

The various preparations of carmine can be used as well for tissues which have been hardened in chromic, picric, or osmic acid, as for fresh tissues, but the former stain less readily.

Picro-nigrosin. Make a saturated solution of picric acid, add crystals of nigrosin, and allow them to dissolve—steep the specimen in it, and allow time for slow staining; this reagent may be used for simultaneous fixing and staining of delicate tissues, and is especially recommended in the preparation of *Spirogyra* and other Algae, and for Fungi.

Potash may either be used in a dilute solution (1-5 per cent) or in a strong solution of water. A dilute solution is commonly used as a clearing agent—it causes cell-walls and starch-grains to swell, especially when heated, and it dissolves sphere-crystals of inulin, crystalloids, and most aleurone-grains, and saponifies fats. It gives a reddish colour to cells in which tannin is present.

A strong solution may be used as a test for suberin, when sections of cork are boiled in strong potash, the suberin escapes in the form of yellow viscid drops, when the sections are only slightly warmed in the solution, the cuticularized walls assume a yellow colour.

A concentrated solution of caustic potash in alcohol is sometimes used with good effect in the preparation of apical meristems, but specimens so treated cannot be permanently kept.

Potassium Acetate (see Acetate).

Potassium Bichromate is used in dilute solution in water as a test for tannin, which it colours dark brown: the 1 per cent. solution in water may also be used for hardening tissues.

Potassium Chlorate is used together with nitric acid as a

macerating agent, and as a test for suberin (see below, Schulze's Macerating Fluid).

Russow's Callus-Reagent (see above, Callus-reagent).

Safranin. This may be used in solution in absolute alcohol. It is especially adapted for staining sections which have been previously hardened with chromic or picric acid. It is not so good for those which have been treated with osmic acid. The sections must be well washed in distilled water, and then placed in a small quantity (1 c.c.) of the saturated alcoholic solution mixed with an equal volume of distilled water; they require to be left for several hours in the staining fluid. They must then be removed, and washed for a short time in alcohol, then they must be placed in absolute alcohol, and kept there until they appear transparent. The sections can now be mounted in distilled water in order to see if the results are satisfactory, or, if they are to be preserved, they must be cleared with oil of cloves, and mounted in Canada balsam or Dammar.

By this means very successful preparations of the structure of nuclei can be obtained.

For double staining with safranin, see Kleinenberg's Hæmatoxylin.

Schulze's Macerating Fluid. Crystals of potassium chlorate may be left to dissolve to saturation in a small bottle of nitric acid, in the cold. The reagent thus prepared is to be used only in small quantities, and the process of maceration should not be conducted in near proximity to microscopes, or other metallic apparatus.

It is used as a macerating fluid for separating the constituents of woody tissues from one another, this result being obtained by the solution of the middle lamella. The tissue to be macerated is cut into small chips, and boiled in a small quantity of the fluid for a short time in a test tube; if the action be too violent, it can at any time be checked by adding water. The fluid is then poured off and the residue collected on a filter, and well washed with water; the specimens may then be mounted in glycerine.

Schulze's Solution (see above, Chlor-Zinc-Iodine).

Sodium Chloride is used as a 10 per cent. solution, or as a saturated solution in water, as a solvent for proteid-crystalloids.

A more dilute solution (1-5 per cent) is used for inducing plasmolysis.

Sulphuric Acid. This is used either concentrated, or dilute (1 to 3 of water). It causes, in either case, the swelling up of cellulose cell-walls, starch-grains, &c. ; when cellulose cell-walls which have been previously saturated with solution of iodine are treated with sulphuric acid, they turn blue.

Concentrated sulphuric acid dissolves cellulose and starch, but cuticularized or corky cell-walls and the middle lamella of lignified cells resist its action. It is used with cane-sugar, as a test for proteids, and a few drops of it are added to a solution of aniline sulphate as a test for lignin.

It may also be used as a solvent for crystals of calcium oxalate.

Turpentine is used with creosote, or carbolic acid, as a clearing agent before mounting in Canada balsam.

Water may be used as a mounting medium, and as a solvent for various reagents ; it may also be used for the cultivation of small organisms, or pollen-grains, spores, or Fungi, under the microscope, and for this purpose a **moist chamber** is to be constructed as follows .—

Several thicknesses of blotting-paper are to be cut to the size of the glass slide, and a circular hole is punched out of the middle, of such a size as to be completely covered by a cover-slip. The blotting-paper is then soaked in water (or boiled in water when pure cultures of Fungi are to be made), so as to saturate it, and placed on the glass slide. A drop of water (or solution as described below) is placed on the cover-slip, the object is immersed in it, and the cover-slip is then inverted over the hole in the blotting-paper. Thus the object is suspended in a drop of liquid on the under surface of the cover-slip. Any loss from the chamber by evaporation is prevented by occasionally wetting the blotting-paper on the slide with freshly boiled, distilled water.

The liquid to be used will of course vary with the nature of the object to be observed. In the case of Algæ, water may be used ; in the case of Fungi, decoctions of various organic substances (fruits, animal tissues, &c.), or a solution of sugar,

according to the habit of the Fungus. For observing the germination of the spores of Mosses and Ferns, water will suffice ; but in the case of pollen-grains a solution of sugar is necessary (1-20 or even 30 per cent., the concentration being different for different plants) ; for observing the process of cell-division in the hairs on the stamens of *Tradescantia*, a 2 per cent. sugar-solution may be used.

APPENDIX B

THIS appendix includes in a tabular form, as being convenient for reference, the more important reactions of the parts of the vegetable cell, and of bodies commonly contained in it.

Cellulose Cell-walls.

- i. Coloured faintly yellow by iodine.
- ii. Swollen and ultimately dissolved by sulphuric acid.
- iii. Coloured blue with iodine and sulphuric acid.
- iv. Coloured blue or violet with chlor-zinc-iodine.
- v. Swollen and dissolved by ammoniacal solution of cupric hydrate.
- vi. Stained by solutions of carmine or of hæmatoxylin which contain a mordant, by methylene blue, and in various degrees by other aniline colours.

Lignified Cell-walls.

- i. Coloured distinctly yellow by iodine, and by chlor-zinc-iodine, but in the case of bast-fibres the tint may vary to sherry brown, or even pink.
- ii. Coloured brown and swollen by iodine and sulphuric acid.
- iii. Coloured bright yellow by acidulated solution of aniline sulphate.
- iv. Coloured red with acid solution of phloroglucin (see Appendix A).
- v. Coloured green when exposed to light after treatment with carbolic and hydrochloric acids (see Appendix A).

vi. Stained slightly or not at all by solutions of carmine, and hæmatoxylin, but readily by aniline colours.

Cuticularized or Corky Cell-walls.

- i. Coloured yellow by iodine.
- ii. Coloured yellow or brown by chlor-zinc-iodine.
- iii. Coloured yellowish by strong potash : on gradually warming (without boiling), they become bright yellow : on boiling, yellow drops of suberin escape.
- iv. They resist the action of sulphuric acid, retaining their clearly-marked outline.

v. On treatment with Schulze's macerating fluid, the cuticularized cell-walls become conspicuous : on boiling in it, the substance escapes as viscid drops of ceric acid.

vi. They are dissolved slowly by strong chromic acid, but resist its action for some time.

vii. They are not stained by solutions of carmine or hæmatoxylin, but are coloured by aniline stains.

Mucilaginous Walls resemble cellulose in many of their reactions.

- i. They swell with water.
- ii. They swell to a greater extent with potash.
- iii. They do not stain with iodine.
- iv. They stain pink with corallin-soda.
- v. They stain red with Hansten's aniline-violet, blue with methylene blue ; some kinds of mucilage also stain with Hoffmann's blue.

Callus is found on the plates of sieve-tubes.

- i. It is soluble in sulphuric acid.
- ii. It is stained by Hoffmann's blue, and by hæmatoxylin
- iii. Brown by Russow's callus-reagent.
- iv. Pink with corallin-soda.
- v. It is largely swollen by potash.

Mineral Deposits in cells or cell-walls.

A. Silica. If a tissue be ignited on platinum foil (after soaking in nitric acid, or Schulze's macerating fluid), and the ash, after being treated with acetic or nitric acid, shows an insoluble residue, the residue is silica.

B. Calcium Oxalate occurs in the form of crystals.

- i. Insoluble in acetic acid.
- ii. Soluble without evolution of gas in nitric acid.
- iii. Soluble in sulphuric acid, with formation of fresh crystals of calcium sulphate, if only small bulk of fluid be present.
- iv. Are not stained with iodine, &c.

C. **Calcium Carbonate** occurs as incrustations, or crystals it is soluble in acetic acid with evolution of bubbles of gas (CO_2).

Protoplasm or Proteids generally.

- i. Coloured yellow or brown by preparations of iodine.
- ii. Coloured yellow by nitric acid : on the addition of potash or ammonia a bright yellow colour is produced (xanthoproteic reaction).
- iii. Swells and loses details of structure on treatment with potash, ammonia, or "eau de javelle."
- iv. Stains readily with solutions of carmine, hæmatoxylin, or Hoffmann's blue ; bright red with Hanstein's aniline violet

The best stains for the nucleus, and for showing the details of its structure, are hæmatoxylin, safranin, and methyl-green.

Plastids show under favourable circumstances the same reactions as other proteid bodies.

Aleurone-grains and crystalloids give also the characteristic reaction of proteids. There is a considerable variety in the solubility of these bodies in water, or in salt-solution, in different seeds : the following will serve as types :—

1. *Grains without crystalloids.*
 - a. Soluble in water : peony, almond, cherry, apple.
 - b. Partially soluble in water ; more or less readily soluble in 10 per cent. solution of common salt.
 - a. Soluble in saturated solution of common salt : lupine, pea, bean, scarlet runner.
 - β. Soluble in saturated solution of common salt only after treatment with alcohol : sunflower, turnip, cress.
2. *Grains containing crystalloids.*
 - a. Partially soluble in water ; more or less readily soluble in 10 per cent. solution of common salt.
 - a. Soluble in saturated solution of common salt . Brazil nut, pumpkin.

- β . Soluble in saturated solution of common salt only
after treatment with alcohol. castor-oil plant, walnut

In all cases a mass (globoid) of mineral matter remains behind after the solution of the grain : this is soluble in acetic acid. The sections should be examined in alcohol.

Starch-grains.

- i. Coloured blue with solutions of iodine in presence of water.
- ii. They swell in solution of potash
- iii. They swell in water above 65°C
- iv. They swell in dilute sulphuric acid.
- v. They swell and are coloured blue with iodine in chloral hydrate.
- vi. They stain pink in corallin-soda solution.

Inulin.

- i. Soluble, but not readily, in cold water
- ii. Precipitated as sphere-crystals on extraction of water by alcohol or glycerine.
- iii. Not appreciably coloured with iodine
- iv. Soluble, without coloration, in potash.
- v. Coloured an orange-red with alcoholic solution of orcin, after warming with hydrochloric acid.

Grape-Sugar.

- i. Soluble in water
- ii. Less soluble in alcohol
- iii. Gives a bulky yellow precipitate with Fehling's solution

Cane-Sugar differs from the above in giving no precipitate with Fehling's solution.

Asparagin.

- i. Soluble in water.
- ii. Precipitated by alcohol.
- iii. Distinguished from other bodies which give the above reaction by insolubility in a saturated solution of asparagin.

Fixed Oils.

- i. Coloured black with osmic acid.
- ii. Saponified more or less readily by potash.
- iii. Soluble in ether.
- iv. Stained pink by alkanna.

v. Some fixed oils are soluble in alcohol. *e.g.* oil of *Ricinus*.

Caoutchouc.

- i. Swollen, but not dissolved, by potash.
- ii. Stained with tincture of alkanet.
- iii. Soluble in chloroform or benzol.

Tannin.

- i. Coloured deep brown by potassium bichromate, or chromic acid.
- ii. Coloured greenish-blue by solution of ferrous sulphate and nitric acid.
- iii. Gives a bulky yellow precipitate with solution of ammonium molybdate in strong solution of ammonium chloride.

Resin.

- i. Soluble more or less readily in alcohol, or ether.
- ii. Coloured red by alkanna.
- iii. Coloured blue by Hanstein's aniline violet.

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